

AN ABSTRACT OF THE THESIS OF

Angie Mestas for the degree of Master of Science in Botany and Plant Pathology presented on December 6, 2018.

Title: Environmental Factors Affecting Phytophthora Root Rot of Rhododendron.

Abstract approved:

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Phytophthora root rot decreases availability and quality of rhododendrons produced in the USA. Symptoms of Phytophthora root rot include root necrosis, leaf chlorosis, stunting, and permanent wilt. The purpose of this thesis was to better understand the impact of root damage, soil moisture, nitrogen fertilizer application, and pathogen species on root rot development.

In chapter 2, a greenhouse experiment with container rhododendrons was conducted to assess if Phytophthora root rot induced by flooding in research studies is representative of disease progression under nursery conditions, whether physical damage to roots increases disease severity, and whether these factors affect root rot caused by *Phytophthora cinnamomi* and *P. plurivora*. Rhododendron 'Boule de Neige' (trial 1) and 'Scintillation' (trials 2 and 3) with either low or high amounts of physical root

damage were grown in a soilless substrate infested with *P. cinnamomi* or *P. plurivora*, then subjected to two water treatments for 18 weeks after inoculation (WAI) (trials 1 and 2) and 24 WAI (trial 3). Plants were either flooded for 48 h then maintained at or below container capacity or not flooded and kept in saucers of water to maintain substrate moisture $\geq 95\%$ container capacity. The flooded treatment is typical of methods used to induce Phytophthora root rot in experimental conditions, whereas the plants in saucers were considered representative of nursery conditions when container plants sit in a shallow pool of water. Root rot and mortality were observed in both the flood and saucer treatment. In general, root rot in plants inoculated with *P. cinnamomi* was more severe than in plants inoculated with *P. plurivora*. There were few differences in disease induced by flooding compared to disease induced by saucers in plants inoculated with *P. cinnamomi*. In contrast, there was more disease in plants inoculated with *P. plurivora* in the saucer treatment compared to the flood treatment. Across all pathogen and water treatments, there were no differences in disease between low and high physical root damage.

P. cinnamomi was generally a more aggressive pathogen than *P. plurivora* and the experimental method of flooding inoculated plants to induce disease produces similar results as placing inoculated plants into a saucer water. It was not necessary to flood for 48 h to induce disease under experimental conditions. However, root rot damage occurred in noninoculated plants of the saucer treatment so future experiments should continue to use the flood treatment to induce disease. Future research should also compare both the flood treatment and the saucer treatment to an inoculation

method that maintains soil moisture around 60 to 70% container capacity, as a high soil moisture content can change soil properties and alter host physiology.

In chapter 3, a greenhouse experiment was conducted to assess the impact of increasing N fertilizer rate on the progression and severity of Phytophthora root rot of rhododendron caused by *P. cinnamomi* and *P. plurivora*. *Rhododendron catawbiense* 'Boursault' was grown with no (0 g N/ pot), low (1.04 g N/pot), and high (3.12 g N/pot) N (urea) incorporated into the container substrate and infested with either *P. cinnamomi* or *P. plurivora* for 18 weeks after inoculation (WAI). Application of N changed plant physiology and promoted greater biomass and leaf greenness when plants were not inoculated with *Phytophthora*. Nitrogen application enhanced N, K, Mg, P, S, and Mn uptake in low and high N treatments. The results suggest that differences in growth between plants in the no N treatment and those fertilized with N were primarily driven by N availability and its influence on the uptake of other nutrients and water. The severity of Phytophthora root rot was greater in plants inoculated with *P. cinnamomi* but not in plants inoculated with *P. plurivora* with increased N. While mortality of plants inoculated with *P. plurivora* was greater from no N to high N, the difference was not significant. Root rot and mortality was greater with added N compared to the no N application rate (from 10 to 50% and 0 to 10% in *P. plurivora* plants in trials 1 and 2 respectively, and from 20 to 70% and 30 to 90% in *P. cinnamomi* plants in trials 1 and 2 respectively). On average, inoculation with *P. plurivora* did not restrict uptake of any nutrient, but inoculation with *P. cinnamomi* restricted uptake of Mg, Mn, and Cu. Although reducing N application would reduce disease severity, the quality of plants infected with either pathogen would be suboptimal as shoot mortality still occurred in the no N treatment.

Decreasing plant N status only slowed the disease. As nurseries may apply high amounts of N during production to promote fast growth and economic production, it is important to understand how N affects growth and disease. In order to understand how N (both rate and form) affects disease, more research is needed on different rhododendron cultivars sensitivity to N and effects of N in vitro on *P. cinnamomi* and *P. plurivora*. The effect of increased N via foliar application should also be examined, as disease severity may not be greater with added N if N is applied away from the site of infection.

In general, plants inoculated with *P. cinnamomi* had more severe disease than plants inoculated with *P. plurivora* in both experiments. Although there was no difference in disease severity as a result of physical root damage, root rot was just as severe, or more severe in the saucer treatment than the flood treatment for *P. cinnamomi* and *P. plurivora* inoculated plants, as well as in the noninoculated control plants. Disease progression and severity was greater with increased N in plants inoculated with *P. cinnamomi* but not *P. plurivora*. Understanding the impact of root damage, soil moisture, nitrogen fertilizer application, and pathogen species on root rot development requires further research. These factors affect disease development and management in the nursery industry and how pathology studies are conducted.

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Environmental Factors Affecting Phytophthora Root Rot of Rhododendron

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TABLE OF CONTENTS

	<u>Page</u>
Chapter 1 General Introduction	1
Chapter 2 Is disease induced by flooding representative of nursery conditions in rhododendrons infected with <i>P. cinnamomi</i> or <i>P. plurivora</i> ?	36
Chapter 3 Biomass of rhododendrons is greater with added N but so is disease severity when plants are infected with <i>P. cinnamomi</i> but not <i>P. plurivora</i>	74
Chapter 4 General Conclusion	112
Bibliography	116

LIST OF FIGURES

Figure	Page
1. Biomass of rhododendron plants (Rhododendron ‘Boule de Neige’ trial 1 and ‘Scintillation’ trial 2 and 3) either not inoculated (Control) or inoculated with <i>Phytophthora cinnamomi</i> or <i>P. plurivora</i> and grown in two different water treatments (Flood and Saucer).....	62
2. Chlorophyll values from leaves on Rhododendron plants (Rhododendron ‘Boule de Neige’ trial 1 and ‘Scintillation’ trial 2 and 3) either not inoculated (Control) or inoculated with <i>Phytophthora cinnamomi</i> or <i>P. plurivora</i> in trial 1 (A), 2 (C), and 3 (E) and grown with two different water treatment (Flood, Saucer) in trial 1 (B), 2 (D), and 3 (F).	63
3. Anthocyanin values from leaves on rhododendron plants (Rhododendron ‘Boule de Neige’ trial 1 and ‘Scintillation’ trial 2 and 3) either not inoculated (Control) or inoculated with <i>Phytophthora cinnamomi</i> or <i>P. plurivora</i> and grown in two different water treatments (Flood, Saucer) in trial 1 (A and B) and averaged across pathogens in trials 2 (C) and 3(D).	64
4. Wilt in rhododendron plants (Rhododendron ‘Boule de Neige’ trial 1 and ‘Scintillation’ trial 2 and 3) either not inoculated (Control) or inoculated with <i>Phytophthora cinnamomi</i> or <i>P. plurivora</i> in trial 1 (A), 2 (B) and 3 (C).	65
5. Mortality in rhododendron plants (Rhododendron ‘Boule de Neige’ trial 1 and ‘Scintillation’ trial 2 and 3) either not inoculated (Control) or inoculated with <i>Phytophthora cinnamomi</i> or <i>P. plurivora</i> in trial 1 (A), 2 (B) and 3 (C).....	66
6. Root rot in rhododendron plants (Rhododendron ‘Boule de Neige’ trial 1 and ‘Scintillation’ trial 2 and 3) either not inoculated (Control) or inoculated with <i>Phytophthora cinnamomi</i> or <i>P. plurivora</i> in trial 1 (A), 2 (B) and 3 (C).....	67
7. Root rot in rhododendron plants (Rhododendron ‘Boule de Neige’ trial 1 and ‘Scintillation’ trial 2 and 3) either not inoculated (Control) or inoculated with <i>Phytophthora cinnamomi</i> or <i>P. plurivora</i> and grown with two different water treatments (Flood, Saucer).	68
8. Total plant biomass of <i>Rhododendron catawbiense</i> ‘Boursault’ plants not inoculated (control) or inoculated with <i>P. cinnamomi</i> or <i>P. plurivora</i> and grown with three rates of nitrogen (N) for 18 weeks after inoculation.	96
9. Chlorophyll (leaf greenness) and anthocyanin (leaf redness) values of <i>Rhododendron catawbiense</i> ‘Boursault’ plants either not inoculated (control) or inoculated with <i>P. cinnamomi</i> or <i>P. plurivora</i> and grown with three rates of nitrogen (N) for 18 weeks after inoculation (WAI) in trial 1 (A and C) and trial 2 (B and D).	97
10. Chlorophyll (leaf greenness) values of <i>Rhododendron catawbiense</i> ‘Boursault’ plants either not inoculated (control) or inoculated with <i>P. cinnamomi</i> or <i>P. plurivora</i> and grown with three rates of nitrogen (N) for 18 weeks after inoculation (WAI) in trial 1 (A, C, E) and trial 2 (B, D, F).....	98

11. Anthocyanin (leaf redness) values of <i>Rhododendron catawbiense</i> 'Boursault' plants either not inoculated (control) or inoculated with <i>P. cinnamomi</i> or <i>P. plurivora</i> and grown with three rates of nitrogen (N) for 18 weeks after inoculation (WAI) in trial 1 (A, C, E) and trial 2 (B, D, F).....	99
12. Stomatal conductance of <i>Rhododendron catawbiense</i> 'Boursault' plants either not inoculated (control) or inoculated with <i>P. cinnamomi</i> or <i>P. plurivora</i> and grown with three rates of nitrogen (N).	100
13. Wilt and mortality in <i>Rhododendron catawbiense</i> 'Boursault' plants either not inoculated (control) or inoculated with <i>P. cinnamomi</i> or <i>P. plurivora</i> and grown with three rates of nitrogen (N).	101
14. Root rot categories of <i>Rhododendron catawbiense</i> 'Boursault' plants either not inoculated (control) or inoculated with <i>Phytophthora cinnamomi</i> or <i>P. pluriovra</i> across both trials.....	103
15. Influence of nitrogen (N) application rate and pathogen inoculation on nutrient uptake (A, B) and leaf (C, D), stem (E, F), and root (G, H) composition in <i>Rhododendron catawbiense</i> 'Boursault' plants either not inoculated (Control) or inoculated with <i>Phytophthora. cinnamomi</i> or <i>P. plurivora</i> and grown with three rates of nitrogen (N).	105

Chapter 1 General Introduction

Host

Importance of nursery industry and economic value of rhododendrons

The 2012 nursery, greenhouse, and floriculture industry accounted for 3.68% (\$14.5 billion) of the total value of agricultural products sold in the United States (USDA National Agricultural Statistics Service 2014). Oregon's industry ranked among the top five states in terms of value of production. In 2016, the nursery and greenhouse industry was the highest valued agricultural commodity in Oregon (Oregon Department of Agriculture 2017). It accounted for 19% (\$909 million) of the top 20 agricultural commodity sales in the state. Within this industry, broadleaf evergreens were valued at \$807 million in the United States and \$70 million in Oregon (USDA National Agricultural Statistics Service 2015). Within this category, over 5% of the national sales (\$42 million) are attributed to rhododendrons. Oregon rhododendron sales account for a quarter of national sales (\$11 million).

The genus Rhododendron

Rhododendrons (*Rhododendron* species and cultivars) are ornamental shrubs (woody plants) primarily grown for their flowers (Sleumer 1980a). Both the cold hardiness of rhododendrons and the range of flower colors make them a popular landscape plant (Roane 1986). Rhododendrons are found naturally in temperate forests throughout Asia and North America with acidic, moist soils, and also in tropical regions of northern Australia, New Guinea, Indonesia, and Malaysia (Bryant 2001).

Rhododendrons belong to the kingdom Plantae, subkingdom Tracheobionta (vascular plant), superdivision Spermatophyta (seed plants), division Magnoliophyta (flowering plants), class Magnoliopsida, subclass Dilleniidae, order Ericales, family Ericaceae (heath family), and the genus *Rhododendron* L. (Natural Resources Conservation Service 2018). Within the genus *Rhododendron* there are many species (approximately 1,000) and varieties (approximately 10,000) (Azalea Society of America 2018). The classification of these species has undergone many revisions and was traditionally divided into 8 subgenera based on morphological characteristics such as flower and leaf morphology: *Rhododendron*, *Hymenanthus*, *Pentanthera*, *Tsutsutsi*, *Azaleastrum*, *Candidastrum*, *Mumeazalea*, and *Therorhodion* (Bryant 2001). With the advent of molecular systematics, a new classification was proposed based on the sequence of a subunit of RNA polymerase II that discontinued and redistributed the subgenera *Candidastrum*, *Pentanthera*, *Tsutsutsi*, and *Mumeazalea* into the other subgenera and created a new subgenus *Choniastrum* (Goetsch et al. 2005). This resulted in five subgenera: *Azaleastrum*, *Choniastrum*, *Hymenanthus*, *Rhododendron*, and *Therorhodion*.

Rhododendrons are sometimes classified in the horticulture industry as either lepidote (having leaf scales) or elepidote (without leaf scales), a taxonomically relevant distinction (Bryant 2001). The subgenus *Rhododendron* contains all lepidote rhododendrons whereas the elepidote rhododendrons are represented by the subgenera *Azaleastrum*, *Choniastrum*, *Hymenanthus*, and *Therorhodion*. Plants in the genus *Rhododendron* were also historically divided into two morphological groups,

rhododendrons and azaleas (Roane 1986). Linnaeus separated the two partly on the basis of the number of stamens, with azaleas having 5 and rhododendrons having 10 or more (Sleumer 1980b). These classifications had inconsistencies, and eventually azaleas were placed into two subgenera within the genus *Rhododendron*: *Pentanthera* (deciduous azaleas) and *Tsutsutsi* (persistent-leaved, or evergreen, azaleas) (Bryant 2001). Differences in inflorescence and seed characteristics, as well as infrequent hybridization, also supported the distinction of *Pentanthera* and *Tsutsutsi* from the other *Rhododendron* subgenera, which are collectively known as rhododendrons (Philipson 1980). This meant that all azaleas were considered rhododendrons, but not all rhododendrons were considered azaleas.

Further reclassification of the genus *Rhododendron* in 2005 (Goetsch et al. 2005) discontinued *Pentanthera* and *Tsutsutsi* because the morphological distinctions were not consistent with the relatedness of the subgenera based on genetics. Under the new classification scheme, *Pentanthera* is now a section in the subgenus *Hymenanthes* and *Tsutsutsi* is now a section in the subgenus *Azaleastrum*. Despite the reclassification of azaleas taxonomically, the common name azalea continues to be used in the horticulture industry to distinguish plants with certain floral and leaf characteristics (e.g. azaleas generally have 5 stamens and their leaves are generally smaller and more pointed than in broadleaf evergreen rhododendrons) (Azalea Society of America 2018).

In experiment 1, the rhododendron cultivars ‘Boule de Neige’ (RHS 58) and ‘Scintillation’ (ARS 734) were used. The ‘Boule de Neige’ seed parent is *R. caucasicum* and the pollen parent is a *R. catawbiense* hybrid. The ‘Scintillation’ seed parent is *R.*

fortunei ssp. *fortunei* and the pollen parent is unknown. In experiment 2, the rhododendron cultivar 'Boursault' (RHS 58) was used. The 'Boursault' seed parent is *R. catawbiense* and the pollen parent is unknown. Both *R. caucasicum* and *R. catawbiense* belong to the subgenus *Hymenanthes*, the section *Ponticum*, and the subsection *Pontica*. *Rhododendron fortunei* ssp. *fortunei* is in the subgenus *Hymenanthes*, section *Ponticum*, and subsection *Fortunea*. All three rhododendron cultivars used for experiments in this thesis are elepidote evergreen rhododendrons.

Rhododendron – propagation, soil moisture, and nutrition

Rhododendrons are propagated by seed, cuttings, grafting, layering, or tissue culture (Hartmann et al. 1990). Of these methods, the principal forms of propagation are from stem cuttings and tissue culture, the latter suited for rapid proliferation of a particular clone (Blazich et al. 1986). Cuttings are generally taken from stock plants and treated with indolebutyric acid (1-2%) to encourage rooting and a fungicide to prevent root rot (Hartmann et al. 1990). After treatment, cuttings are placed into a substrate to root. A typical substrate for cutting propagation consists of 2/3 sphagnum peat moss and 1/3 perlite. Cuttings are kept moist to encourage rooting. Once roots are established, cuttings are transplanted into larger containers, sold, or transplanted to the field for production of larger plants.

Tissue culture propagation begins with the establishment of soft wood shoots from stock plants in culture (2-3 months), followed by multiple cycles of shoot multiplication (6 months). Tissue culture media vary in composition, but often include inorganic salts to provide essential nutrients, organic compounds to provide

carbohydrates, vitamins, hormones and growth regulators to promote cell multiplication. Inert ingredients like agar and liquid media add physical supports, and the suspension may be placed on a shaker to provide aeration. After shoot multiplication, shoots are rooted in either soil (1-2 months) or culture media (1 month). Rooted plants are then placed in liners containing a growing substrate until established (3 months). At this point, plants can be transplanted to larger containers and sold.

In general rhododendron nursery plants require growing substrates that are moist and have good drainage (Shelton 1967). Although nurseries grow some larger rhododendrons in fields, most are container-grown in soilless substrates. Examples of substrate components used in container production include peat moss (for its high water holding capacity, acidic pH, and high organic matter), bark (to increase aeration) and perlite (to increase air porosity), slow release nitrogen (N), dolomitic limestone (pH buffer) and a wetting agent (Bunt 1988; Larson 1980).

In general rhododendrons require substrates with moderately low solute concentrations (Larson 1993) and low pH (pH 4.5 to 6) (Shelton 1967). To avoid injuring rhododendron roots it is important to maintain moderately low EC and low pH. High salinity (1) decreases water potential outside the plant leading to water stress and (2) overwhelms the capacity of the vacuoles to store salts which prohibits movement of carbohydrates and hormones needed for growth (Kozlowski 1997). The optimum range of electrical conductivity, which is a measurement of solute concentration, for containerized rhododendron production is between 0.1-1 dS/m (Rouse 1984) and may be as high as 2 dS/m (Cooke and Bilderback 1995). Levels above 2 dS/m may damage

rhododendron roots if the irrigation volume is not adjusted to leach salts from the root zone. Although some rhododendron species are tolerant of neutral to basic pH (6.0, 7.5 and 9.0), including *R. macrosepalum* var. *hanaguruma* and *R. scabrum*, high pH generally limits growth and leads to chlorosis due to iron deficiency (Scariot et al. 2013). In nursery production, fertilizer applications are one of the primary drivers of substrate EC and pH.

Nitrogen (N) promotes plant growth and is an essential component in the production of proteins, hormones, phytoalexins, phenolics, and enzymes (Huber and Thompson 2007). Nitrogen can be taken up by plants in an oxidized or reduced form. For rhododendrons N should be applied as ammonium (NH_4^+) (Clark et al. 2003). Fertilizers with NH_4^+ , which lowers pH, produce larger rhododendrons with an overall higher plant quality compared to fertilizers with nitrate (NO_3^-), which produce smaller plants with chlorotic leaves. The form of N also affects foliar concentrations of other essential nutrients, it is therefore important to consider use of supplemental minerals. *Rhododendron austrinum* and *R. canescens* 'Brook' supplied with NH_4^+ had lower foliar concentrations of N, potassium (K), calcium (Ca), sulfur (S), boron (B), and molybdenum (Mo) compared to plants supplied NO_3^- . The normal range of elements in foliar tissue of azaleas are: 2-3% N, 0.29-0.50% phosphorous (P), 0.80-1.60% K, 0.22-1.60% Ca, 0.17-0.50% magnesium (Mg), 30-300 ppm manganese (Mn), 50-150 ppm iron (Fe), 6-15 ppm copper (Cu), 17-100 ppm B, 5-60 ppm zinc (Zn) and less than 1500 ppm sodium (Na) (Larson 1993).

Irrigation management influences production efficiency and product quality in container production of rhododendrons (Koniarski and Matysiak 2013). Vegetative growth and flowering is improved by reducing irrigation volume and modifying irrigation frequency (Beeson 1991; Cameron et al. 2015; Koniarski and Matysiak 2013; Scagel et al. 2014; White 1937). Rhododendrons that are well-watered have a volumetric water content between 0.4 to 0.5 m³ m⁻³, corresponding to a medium matric potential of roughly -1.5 to -2.6 kPa (Cameron et al. 2015). Rhododendrons that are produced under moderate drought have a volumetric water content between 0.15 to 0.30 m³ m⁻³, corresponding to a medium matric potential of roughly -3.4 to -11.2 kPa.

Irrigation based on maintaining a water deficit is called regulated deficit irrigation (RDI) and is designed to improve both water use and plant quality (Koniarski and Matysiak 2013). Rhododendron 'Catawbiense Boursault' and 'Old Port' irrigated at 100% pan evapotranspiration (ET_p), which is the volume of water to balance water loss due to evapotranspiration, exhibited uneven growth. Compared to plants irrigated at 100% ET_p, plants produced at lower irrigation volumes, 50% ET_p (strong deficit irrigation) and 75% ET_p (moderate deficit irrigation), exhibited more compact, uniform growth, with smaller, uniformly sized leaves, and comparable numbers of inflorescence buds. Similarly, Rhododendron 'Hoppy' irrigated at 75% ET_p (moderate drought) had comparable growth to plants irrigated at 1.5X ET_p (well-watered) (Cameron et al. 2015).

Different application frequencies of the same daily volume of irrigation water also affects vegetative and reproductive growth (Scagel et al. 2014). Once daily irrigation resulted in increased vegetative growth and decreased reproductive growth of 'Gibraltar'

(a deciduous rhododendron), and decreased vegetative growth and improved flowering of 'P.J.M. Compact' (an evergreen rhododendron), and 'English Roseum' (an evergreen rhododendron) compared to plants irrigated the same volume split in two applications (Scagel et al. 2014). In another study, *R. 'Fashion'* plants irrigated twice or more per day to maintain field capacity grew more quickly than plants irrigated once daily at the rate of 0.25 inches per day (average nursery rate) (Beeson 1991).

Results from several studies suggest that RDI and modifying irrigation frequency will help in achieving desired vegetative and floral qualities in Rhododendron. Another way that water volume is regulated and measured is by the use of container capacity measurements. Container capacity is the percentage of pore space occupied by water in a substrate that has been drained following saturation (Nemali 2017). In a study that compared maximum growth of *Rhododendron ponticum* at 30, 40, 50, 60, 70, 80 and 90% container capacity, maximum growth was observed at 60 to 70% container capacity (White 1937).

Pathogen

The genus Phytophthora, oomycete

The genus *Phytophthora* encompasses a group of organisms that were once grouped in the kingdom fungi and continue to be referred to as fungus-like (Erwin and Ribeiro 1996a). Today, they are known to be different from true fungi and are more closely related to brown algae. *Phytophthora* differ from true fungi in their evolutionary phylogeny, morphology, reproductive strategies, metabolic pathways, and molecular biology systems. Unlike true fungi, *Phytophthora* and other oomycetes have: (1) a

vegetative thallus that is diploid rather than haploid; (2) mitochondria with tubular instead of flattened cristae; (3) biflagellate heterokont zoospores; (4) cellulose and β -1-3 glucans instead of chitin in their cell walls; (5) do not convert squalene to sterols; (6) have mycolaminarins, a unique storage compound different from true fungi; and (7) produce lysine via the diaminopimelic acid pathway, the same pathway as in plants. The features which distinguish *Phytophthora* from true fungi have been utilized to improve isolation, identification, and management of diseases caused by these organisms. For example, polyene antibiotics are used to isolate *Phytophthora* species from environmental samples because they inhibit the growth of most true fungi.

The genus *Phytophthora* belongs to the kingdom Straminipila (formerly Chromista), phylum Oomycota, order Peronosporales, and family Peronosporaceae (McLaughlin and Spatafora 2014). The recognized species are grouped into 10 clades (Kroon et al. 2012). These clades are based on morphology and on multiple molecular sequences including: internal transcribed spacers 1 and 2 (ITS1 and ITS2), 5.8S ribosomal RNA, and mitochondrial or nuclear “housekeeping genes” involved in metabolism i.e. cytochrome oxidase I and II (*cox1* and *cox2*). The clades show the relatedness of species and the evolution of the genus. The first reported *Phytophthora* species was *Phytophthora infestans*, since then, there have been more than 100 recognized *Phytophthora* species (Kroon et al. 2012).

The lifecycle of *Phytophthora* consists of both a sexual and asexual stage (Erwin and Ribeiro 1996a). Some species of *Phytophthora* are self-fertile (homothallic) while others are self-sterile (heterothallic) and consist of mating types A1 and A2. The sexual

structures are the antheridium (male gametangium) and oogonium (female gametangium) which are the sites of meiosis and produce haploid gametes. During fertilization, the antheridium either encircles the oogonium (amphigynous) or attaches to the side of the oogonium (paragynous). A fertilization tube from the antheridium then deposits an antheridial nucleus (male gamete) into the oogonium where it fuses with the female gamete, the oosphere. The resulting fertilized oosphere, is called an oospore and is diploid. Upon germination, the oospore can either form sporangia or hyphae. Hyphae are tubular filaments that comprise mycelia, which is the thallus, or body, of the organism. Sporangia and mycelia are part of the asexual stage of *Phytophthora*. Sporangia can later produce either mycelia, chlamydospores, or zoospores. When sporangia produce zoospores, they are called zoosporangia. Zoospores are asexual, biflagellate, infectious spores that swim to host roots where they encyst, germinate, and form mycelia that penetrate and infect the roots. Zoospores are produced quickly in response to increased soil moisture and are often produced in large numbers, resulting in increased inoculum levels. Chlamydospores are thick walled asexual spores that function as survival structures.

Effects of water on Phytophthora biology

Water is one of the most important environmental factors that affect both the growth, development, and survival of *Phytophthora* species. Water plays an important role in enhancing the formation and direct (germ tube formation) and indirect (zoospore release) germination of sporangia (Duniway 1983). In culture, *P. citricola* formed the greatest number of sporangia at matric potentials between -5 to -7.5 kPa and produced

the most oospores below a matric potential of -2.5 kPa (Hardy and Sivasithamparam 1991). In another study, sporangia of *P. cinnamomi* were produced in soil at matric potentials between -1 to -250 kPa with the most sporangia produced at -16 kPa (Gisi et al. 1980). When mycelia were placed on the soil surface, the greatest number of sporangia were produced at +0.1 kPa (flooded conditions) and 0 kPa (saturated conditions). In soils with 5 to 50% soil water content, *P. cinnamomi* sporangia and zoospores were only observed at 50% water content, but chlamydospores were produced across the entire range of soil water content (5 to 50%) (Reeves 1975). In another study with *P. cinnamomi*, hyphal lysis occurred most rapidly when soils were at either field capacity or 50% field capacity, and slowest when soils were maintained at twice field capacity (Nesbitt et al. 1979). This study also showed that sporangia formation was greatest in soils at field capacity. Research suggests that *P. cinnamomi* can produce sporangia across the range of water potentials found in soils under production (0 to -400 kPa) (Duniway 1983; Welsh and Zajicek 1993). Outside of the optimal range of sporangia production of *P. cinnamomi* (-80 to -1050 kPa water potential), mycelia can persist and serve as a source of inoculum (Malajczuk and Theodorou 1979).

Effects of nitrogen on Phytophthora biology

The growth and development of *Phytophthora* species also relies on availability of carbon, nutrients, and vitamins. *Phytophthora* species are heterotrophic (require an organic source of carbon) and nutritional requirements vary among species (Hohl 1983). Although some nutrient sources enhance growth more than others, *Phytophthora*

species metabolize a variety of vitamins and nutrients. The only essential vitamin for the growth of *Phytophthora* is thiamin (vitamin B₁). There are multiple sources of nutrition for *Phytophthora* species in nursery production of woody ornamentals, including sugar exudates from plant roots and nutrients commonly applied in fertilizers. The concentrations of Ca, Mg, K, and Fe, that resulted in the largest number of *P. cinnamomi* zoosporangia in vitro were 0.71 mM, 0.16 mM, 0.62 mM, and 0.11 mM respectively (Halsall and Forrester 1977). However, the optimal concentration of these cations for formation of zoosporangia is not known for many *Phytophthora* species, including *P. plurivora*.

Many species metabolize inorganic N sources (e.g. NH₄⁺ and NO₃⁻), but generally have improved growth with organic N sources. In comparison of *P. cinnamomi* growth rates (mm 24h⁻¹) and colony fresh weights (mg) between inorganic N ((NH₄)₂SO₄) and organic N (asparagine), growth rate and colony fresh weight was generally greater with organic N across osmotic potentials of 0 to -2.5 MPa (Luard 1985). Asparagine meets the N requirement for most species (Hohl 1983) and many studies report that an organic source of N is necessary for sexual reproduction. *P. cinnamomi* had greater mycelium dry mass when grown with N in the form of nitrate (0.290 g) versus N in the form of urea (0.230 g) or ammonium (0.225 g) (Duvenhage et al. 1992). There is preliminary evidence that *Phytophthora* species belong to three groups that differ in their ability to assimilate N: group 1 assimilates nitrate efficiently, group 2 assimilates ammonium and organic N efficiently, and group 3 requires organic N. For example, *P. fragariae* belongs to the group that requires organic N as it has lost

its ability to efficiently assimilate oxidized N (NO_3^-). Specific nutritional requirements of *P. plurivora*, and closely related *P. citricola*, are not known.

Phytophthora pathogens in nurseries

Many *Phytophthora* species have been isolated from rhododendrons during surveys of nursery pathogens from a local to a worldwide scale (Kroon et al. 2012; Leonberger et al. 2013; Rytönen et al. 2012). In a survey of ornamental nurseries and gardens in the Czech Republic, *P. plurivora* was isolated from diseased leaves, branches, collars, and roots of multiple ericaceous plants including *Rhododendron catawbiense* 'Grandiflorum,' *R. 'Cunningham's White'* and others (Mrázková et al. 2011). In a 2006-2009 survey of four Oregon wholesale nurseries 28 *Phytophthora* taxa were identified from *Rhododendron*, *Pieris*, *Kalmia*, and *Viburnum* plants, potting material, soil, and water samples taken from production sites (Parke et al. 2014).

Phytophthora cinnamomi and *P. plurivora* were the most commonly isolated species. Eleven taxa were isolated from symptomatic leaves including *P. syringae*, *P. plurivora*, *P. pini/citricola* III, *P. taxon Pgchlamydo* (later described as *P. chlamydospora*), *P. hibernalis*, *P. Marion-2012*, *P. riparia*, *P. bilorbang*, *P. cactorum*, *P. hedraiaandra*, and *P. lacustris*-like during a study of Oregon nurseries in 2011-2012, that focused specifically on the genus *Rhododendron* (Knaus et al. 2015). *Phytophthora syringae* and *P. plurivora* were the most commonly isolated species. The co-occurrence of root rot with *P. plurivora* and *P. cinnamomi* from ornamental nurseries suggest that these two pathogens may play an important role in disease.

Phytophthora cinnamomi

Phytophthora cinnamomi was first described in 1922 by Rands on cinnamon trees, *Cinnamomum burmanni* Blume, in Sumatra, Indonesia (Zentmyer 1983). It is in clade seven of the *Phytophthora* genus (Kroon et al. 2012). The majority of clade seven species are pathogenic on roots and have nonpapillate zoosporangia. *P. cinnamomi* is reported as pathogenic in many natural and agricultural settings and has a broad host range (Beaulieu et al. 2017; Duan et al. 2008).

Phytophthora cinnamomi is heterothallic (Kroon et al. 2012). It reproduces asexually by producing chlamydospores, sporangia, and zoospores, and sexually by producing oospores. However, there is no evidence of sexual reproduction in the United States (Beaulieu et al. 2017; Duan et al. 2008). The dominant mating type globally and in the US is A2 and it reproduces clonally. Morphologically the antheridia (male gametangium) has amphigynous (encircling) attachment to the oogonium (female gametangium) (Kroon et al. 2012). The sporangia are nonpapillate (lacking developed papilla or rounded protuberance). *Phytophthora cinnamomi* is most active in warm temperatures (16-24°C) and water logged soils (Burgess et al. 2017; Fraher 2014).

P. cinnamomi is commonly isolated from infected roots and stems of rhododendrons. The pathogen affects plants of 260 genera of 90 plant families including *Rhododendron*. Since the 1930s, *P. cinnamomi* has been considered a serious pathogen of rhododendrons nationally (White 1930).

Phytophthora plurivora

P. citricola was originally described in 1927 (Zentmyer 1983). The earliest report of *P. citricola* on rhododendron was 1969 (Hoitink and Schmitthenner 1969). Since then,

P. citricola has been identified as a complex and broken into multiple *Phytophthora* species: *P. citricola sensu stricto*, *P. menzei*, *P. capensis*, *P. elongata*, *P. multivora*, and *P. plurivora* (Kroon et al. 2012). These species are in clade two of the *Phytophthora* genus (Kroon et al. 2012). The majority of clade two species are homothallic (15/21), including *P. plurivora*, and clade two species have zoosporangia that are either papillate or semipapillate. Population genetics has shown that Europe is the center of origin for *P. plurivora* and that the pathogen was introduced to the U.S., likely via nursery trade (Schoebel et al. 2014). Like *P. cinnamomi*, *P. plurivora* has been reported as pathogenic in many natural and agricultural settings (Schoebel et al. 2014). It is commonly isolated from infected roots and stems as well as from baits from rhizosphere soil (Burgess and Jung).

Phytophthora plurivora is able to reproduce both sexually by oospore production and asexually by sporangia and zoospores (Jung and Burgess 2009). Sequence data from population genetics support that the pathogen is homothallic (Schoebel et al. 2014). Therefore, sexual reproduction is not limited by absence of an opposite mating type. Morphologically the antheridia (male gametangium) has paragynous (side) attachment to the oogonium (female gametangium) (Kroon et al. 2012). The sporangia are semipapillate (less well-developed papilla or rounded protuberance). On many hosts in nurseries it is most active in summer as warm temperatures favor the disease (Grunwald 2014). Like *P. cinnamomi*, the pathogen affects a range of woody hosts including rhododendron. It affects both foliar and root tissue (Kroon et al. 2012).

Rhododendrons are considered an important host in the movement and spread of *P. plurivora* (Lilja et al. 2011; Schoebel et al. 2014). The pathogen is frequently isolated from symptomatic rhododendrons in nurseries and spread is increasing due to trade (Schoebel et al. 2014).

Phytophthora root rot

Phytophthora root rot, caused by multiple *Phytophthora* species, is a common nursery disease of rhododendrons that causes significant production losses (Englander et al. 1980; Hoitink et al. 2014). Typical *Phytophthora* species that cause root rot include *P. cinnamomi*, *P. cambivora*, *P. nicotianae*, *P. cryptogea*, and *P. citricola* (Hoitink et al. 2014). Later studies have shown that *P. plurivora* is also common (Parke and Grunwald 2012). It may be that *P. citricola* isolates reported by Hoitink were actually *P. plurivora*, as *P. plurivora* was only recently described as a morphologically similar, but cryptic species within *P. citricola* sensu lato (Jung and Burgess 2009). These pathogens are soil-borne organisms that infect the roots and cause root necrosis. Damage to roots inhibits adequate water and nutrient uptake, leading to above ground symptoms of water and nutrient stress, including leaf curl, leaf chlorosis, shoot wilt, and shoot necrosis. The disease is most problematic in 1-2 year old plants, especially those produced in containers (Hoitink et al. 2014).

Loss from Phytophthora root rot has increased as container production has increased (Englander et al. 1980). In a survey of 10 North Carolina nurseries, incidence of Phytophthora root rot ranged from 1 to 19% (Benson et al. 1982). In Ohio, losses attributed to *P. cinnamomi* ranged from 10-15% (Hoitink and Schmitthenner 1969). In

another survey, losses in nursery stock ranged from 1 to 50% due to infection by *P. cinnamomi* and *P. citricola* (Orlikowsky and Szkuta 2008). *Phytophthora* root rot is considered one of the most damaging diseases in commercial nurseries and many cultivated varieties of ericaceous plants are susceptible to *Phytophthora* pathogens (Englander et al. 1980; Mrázková et al. 2011).

Effects of water on Phytophthora root rot

High water environments exacerbate *Phytophthora* root rot (Atkinson 1965; Duniway 1983; Wager 1942). Early reports of root rot on avocado trees were initially attributed to water injury and lack of aeration, as plants with the most severe symptoms were in areas with high soil moisture (Wager 1942). However, *P. cinnamomi* was later isolated from the roots of symptomatic plants and subsequent pathogenicity studies using *P. cinnamomi* isolates developed wilt and root rot when grown in high soil moisture. In another early report, *Phytophthora cambivora* (Petri) Buis was identified causing severe disease on rhododendrons in depressions where excess irrigation water pooled and disease was also observed to spread down slopes (White 1937). When *Rhododendron ponticum* was inoculated with *P. cinnamomi* and plants were grown across a range of soil moistures (30 to 90% container capacity), symptoms first appeared on plants held at high soil moistures (White 1937). It is now understood that high soil moisture alone does not lead to disease but that water plays an important role in the spread of the infectious, disease causing propagules of *Phytophthora* (Erwin and Ribeiro 1996b).

Because of the importance of water in the spread and germination of sporangia, as well as infection, flooding for 24 to 48 h is commonly used in studies to induce disease (Browne and Viveros 1999; Parke 2007; Weiland et al. 2010) but flooding rarely occurs in container nurseries. Disease severity of Mahaleb and Mazzard cherry seedlings (*Prunus avium* L. 'Silverbark Mazzard') inoculated with *P. cryptogea* and *P. megasperma* increased from 0 to 8 h of flooding and from 8 to 16 h but only increased from 16 to 24 h in Mahaleb seedlings inoculated with *P. cryptogea* (Wilcox and Mircetich 1985).

In a comparison of disease in blueberry plants (*Vaccinium corymbosum* hybrid) inoculated with *P. cinnamomi* that were flooded for 24 h or 48 h or not flooded, mortality was highest in plants flooded for 48 h (Smith et al. 2017). In a study comparing disease in apple seedlings (*Malus pumila* Mill) inoculated with *P. cryptogea*, *P. cactorum*, and *P. cambivora*, that were flooded 0, 4, 12, 24 or 48 h, disease severity increased with flooding duration only for plants inoculated with *P. cryptogea*, in which disease severity increased from mild (1% root rot) in 0 h flood to severe (52% root rot) in 48 h flood (Browne and Mircetich 1988). Maximum zoospore activity (as detected by baiting) was observed in the shorter flood durations (0 to 4 h) for *P. cactorum* and *P. cambivora* and in the longer flood durations (44 to 28 h) for *P. cryptogea*.

Early Phytophthora root rot studies created and maintained high moisture by using simple automatic drip watering systems (e.g. glass wick method and plastic capillary tube method) (Atkinson 1965). Using these types of watering systems, symptoms appeared 20 days following inoculation by *P. cinnamomi* when containerized

Chamaecyparis lawsoniana Parl. were watered every 30 min with 80 to 105 ml or 95 to 130 ml, respectively using the glass wick or plastic capillary method. Another method previously used to maintain high soil moisture in research of *Phytophthora* root rot was the use of water filled saucers placed underneath containers (Tsao and Garber 1960). This method provided a suitable environment for root infection but also has potential to impair growth of noninfested plants. The high water environments of these studies are meant to mimic conditions found in commercial nurseries in which there is excessive water from heavy rains, faulty irrigation, or poor drainage below containers (Blaker and MacDonald 1981). These conditions are commonly associated with high disease loss in nursery plants.

Effects of nitrogen on Phytophthora root rot

Nitrogen fertility may play an important but differential role in disease. Some studies show that high N increases disease severity by altering host tissue to make it more easily penetrable by pathogens (Tan et al. 2002; Utkhede and Smith 1995). Mortality of durian (*Durio zibethinus* Murr.) inoculated with *P. palmivora*, which causes root rot in durian, increased with N rate (Tan et al. 2002). Severity of crown and root rot of apple trees (*Malus domestica* Borkh.) inoculated with *P. cactorum* increased with added N (Utkhede and Smith 1995). In Utkhede and Smith's (1995) study, high N was thought to increase disease severity because plant tissue becomes more succulent with N application, which favors pathogen penetration. There are also reports that high plant N status has no effect or decreases disease (Lee and Zentmyer 1982; Scheuerell et al. 2005; Zentmyer and Bingham 1956). Some studies suggest that N decreases inoculum

while another suggests that N enhances host defense responses. Increased N increased suppression of damping-off, which causes seedling collapse, in various rootstocks inoculated with *Pythium ultimum* (Scheuerell et al. 2005). Increased N decreased root rot in *Persea indica* inoculated with *P. cinnamomi* (Lee and Zentmyer 1982). In Lee's study, high N is thought to lower disease because high N decreases inoculum level. Root rot severity of avocado inoculated with *P. cinnamomi* decreased with added N (Zentmyer and Bingham 1956). In Zentmyer and Bingham's (1956) study, high N reduced inoculum. There is no information on the effect of elevated N on disease caused by *P. plurivora*. However, in beech seedlings (*Fagus sylvatic*) inoculated with *P. citricola*, a *Phytophthora* species closely related to *P. plurivora* that causes root rot, mortality decreased with high N (Fleischmann et al. 2010). In Fleischmann's study, while high N increased pathogen sporulation in vitro slightly, high N is thought to promote defensive compounds in the plant.

The form of N may also affect disease severity (Huber and Watson 1974; Utkhede and Smith 1995). Root rot severity of avocados (cv Edranol) inoculated with *P. cinnamomi* was greater with N applied in the form of urea (rating of 5) and nitrate (rating of 4.9) versus with N applied in the form of ammonium sulfate (rating of 4.1) where root rot rating of 5 = root rot symptoms on more than 80% of root area and 4 = root rot symptoms on 61 to 80% of root area (Duvenhage et al. 1992). Citrus seedlings inoculated with *P. citrophthora* or *P. parasitica* had increased susceptibility with N applied as ammonium sulfate or urea (47 to 59% of roots infected) than with N applied as nitrate (3% of roots infected) (Huber and Watson 1974). Crown and root rot severity

of apple trees (*Malus domestica* Borkh.) inoculated with *P. cactorum*, increased with added N in the form of ammonium nitrate, and was unaffected with added N in the form of monoammonium phosphate (Utkhede and Smith 1995). Changing the form of N will have little effect if the host is highly resistant or highly susceptible (Huber and Watson 1974).

Other chemical attributes of the root environment, such as pH and EC, can alter Phytophthora root rot. In general, it is believed that increasing acidity (lower pH), decreases disease caused by *P. cinnamomi* and other *Phytophthora* species (Schmitthenner and Canaday 1983). While disease was high at pH 4-7, disease was reduced at pH below 4 in *R. ponticum* inoculated with *P. cambivora* (White 1937). This cultivar tolerated a pH below the recommend pH range for many rhododendron species (Shelton 1967). Maintaining a pH below 4 to control disease is not feasible as the general pH requirement for rhododendron production is 4.5 to 6. Survival, growth, sporulation and infection of rhododendron by *P. ramorum* is negatively correlated with increased salinity. While rhododendron leaf discs were most often infected at the lowest solute concentration, 5.6 mS, infection occurred in salt concentrations up to 57.2 mS (Preuett et al. 2013), which is within range of the recommended salt concentration for rhododendron production (50 to 200 mS/m) (Cooke and Bilderback 1995).

Rhododendron roots and *Phytophthora* root rot pathogens have some similar environmental parameters for growth, including high moisture, low pH, low solute concentrations, and ability to utilize N in the form of NH_4^+ . Experiment 2 in this thesis examined how increasing levels of N in the form of NH_4^+ , which lowers pH, alters

disease severity in container grown rhododendrons inoculated with *P. cinnamomi* and *P. plurivora*.

Disease control

Fungicides are the most common control method of *Phytophthora* diseases (Beaulieu et al. 2017). Fungicides used to prevent *Phytophthora* applied as drenches and sprays include fosetyl aluminum, mefenoxam, and etridiazole (Hoitink et al. 2014). The fungicides: Biophos 43L (phosphorous acid), Vital 4L, Aliette 80W (fosetyl aluminum), Cyazofamid 400SC (cyazofamid), Fenstar 500SC (fenamidone), and Stature DM 50W (dimethomorph, mandipropamid) applied preventatively at the label rate, prior to infection by *P. cinnamomi*, prevent root rot on azalea through the growing season without causing phytotoxicity (Benson and Parker 2005). Truban (terrazole) and Subdue MAXX (mefenoxam) applied at the label rate failed to prevent root rot through the growing season. The fungicide metalaxyl applied to rhododendrons prior to inoculation with *P. cinnamomi* moderately controlled disease and was more effective than ethazole (Englander et al. 1980).

Other control methods include biological control, host plant resistance, and cultural control (Hoitink et al. 2014). Biological control is disease suppression from plant natural products and/or beneficial microorganisms. Biological properties in the substrate that are associated with less disease include inhibitors of zoospore and sporangia from composted tree bark (composted at 37°C to 54°C for 10 weeks) and antagonistic effects of specific *Trichoderma*, *Pseudomonas* and *Bacillus* species (Hoitink et al. 2014; Reeves 1975). In vitro studies show that *Trichoderma viride* is antagonistic to growth of

P. cinnamomi (Reeves 1975). *Pseudomonas fluorescens* inhibits growth of *P. cinnamomi* in vitro (Santoyo et al. 2010). A strain closely related to *Bacillus acidiceler* inhibits growth of *P. cinnamomi* in vitro (Mendez-Bravo et al. 2018).

Disease resistance is the use of resistant cultivars rather than susceptible cultivars and is based on genetic differences in susceptibility. Most rhododendron cultivars produced commercially are highly susceptible to *P. cinnamomi* and those that are resistant can become susceptible under extreme soil moisture conditions (Blaker and MacDonald 1981). For example, both 'English Roseum' and 'Caroline' are considered relatively resistant, but can become susceptible when exposed to high soil moisture (e.g. after a 48 hour flood) (Blaker and MacDonald 1981; Hoitink and Schmitthenner 1974). Both 'English Roseum' and 'Caroline' are elepidote rhododendrons. Lepidote rhododendrons are considered to be more resistant than elepidote rhododendrons to *Phytophthora ramorum* (Dobbelaere et al. 2010).

Cultural control practices can also be used to reduce the chances for root rot to develop. Chemical and physical properties of the growing substrate that are associated with less disease include: high total porosity (68-86%), high air space (25-36%), low bulk density, which is inversely related to porosity (0.15-0.53 g/cm³), low matric potential -5.0 to -10.0 kPa (Ownley et al. 1990), and a pH < 4 which is suboptimal for *P. cambivora* growth (White 1937). Other production practices that may reduce disease include sanitation, use of pathogen free cuttings, production materials and irrigation water, and placement of containers on surfaces not prone to flooding (Coffey 1987). In addition, avoiding root damage and avoiding use of ammonium nitrate, may also help to

reduce disease. Root damage can affect the plant's ability to transport water, resulting in drought stress (Aldahadha et al. 2012) that may further predispose plants to infection (Blaker and MacDonald 1981). High salts also damage roots and lead to more disease (Moorman 1986). Crown and root rot severity increased with added N in the form of ammonium nitrate, and was unaffected with added N in the form of monoammonium phosphate (Utkhede and Smith 1995).

Nursery production practices and root rot

Irrigation source and management in nursery production can be very specific to each nursery (Hartmann et al. 1990). Water sources for nursery irrigation include well water and surface water. Methods for disinfecting recycled irrigation water vary among nurseries. Many nurseries disinfect irrigation water, especially recycled water, with chemicals to remove pathogens (Hong and Moorman 2005). Common chemicals used to disinfect water include chlorine, ozone, peroxide, copper, and silver. Irrigation water can also be disinfected using ultraviolet light. Efficacy of water disinfection treatments can vary with duration of exposure and concentration. *Phytophthora* spp. can be removed from nursery irrigation water containing 0.77 mg l⁻¹ chlorine (Hong and Kong 2003).

Irrigation methods commonly used in nursery production include hand watering, overhead sprinklers, and/or dripline irrigation. Frequency and volume of water used in containerized production vary by nursery and depends on many factors such as plant type, availability, cost, substrate, container, fertility program and weather. It is important to keep in mind that irrigation frequency and volume affects nutrient availability in the

substrate (Scagel et al. 2011a). Plants can easily be over-watered resulting in standing water, especially when irrigation is not based on growth needs or soil moisture. In addition to standing water from over-irrigation, excess water can also result from heavy rains, broken irrigation systems, poorly designed system coverage, and poor drainage. These situations result in puddling and only rarely cause flooding.

Growers use a range of fertilizers in nursery rhododendron production (Shelton 1967). Fertilizers vary in their nutrient composition (e.g. concentrations, ratios and forms of nutrients), mode of release (liquid feed versus slow release), rates (low to high) and application timings (weekly versus monthly). Differences among fertilizers result in differences in plant growth, substrate pH and EC. Specially formulated commercial fertilizers are available for rhododendrons, such as the N-P-K mixes 4-8-8 and 4-12-12. Volume and frequency of application depend on cultivar, time of year, plant age, container type, irrigation, form of fertilizer, and size of the plant (Shelton 1967). Powdered sulfur or ammonium sulfate can be applied to increase acidity if necessary as rhododendrons are acid-loving plants. Micronutrient fertilizers commonly used include iron, magnesium, zinc, manganese, cobalt, and copper, all of which are water soluble. These compounds, called soluble salts, can be monitored with electrical conductivity readings. Less information is available for optimum fertility management for containerized production than field production that includes standards for fertility composition, rate, application timing, and frequency. Nurseries have to rely on trial and error and monitor substrate pH and EC as well as plants for visual symptoms of salt damage (Cooke and Bilderback 1995). Plant N can be monitored with leaf color

readings, nutrient analysis, and growth measurements (Clark et al. 2003; Scagel et al. 2011b). Problems of both N leaching and over-fertilization in rhododendron production have been reported (Alt et al. 1994; Colangelo and Brand 2001).

Nursery production practices can damage roots intentionally or by accident. Roots provide support, water and nutrient uptake and may be damaged in various ways during production (Dong et al. 2003). Roots may be damaged in a controlled manner to improve plant quality and increase growth, a technique referred to as root pruning, or in an uncontrolled manner during transplanting. These injuries impede water and nutrient uptake. These damages may play a role in predisposing plants to increased disease and create points of entry for pathogens (Coyier 1980; White 1937). In addition to physical damage from transplanting and pruning, roots may also be damaged from heat stress. Roots exposed to high temperatures of 40°C and 50°C for 20 minutes were more often infected by *Phytophthora parasitica* Dastur than roots exposed to lower temperatures of 22°C and 30°C (Lyles et al. 1992). Root rot caused by *Phytophthora cypogea* was more severe in roots exposed to temperatures above 45°C for 30 minutes than roots at 25°C or 35°C (MacDonald 1991).

The nursery environment is part of the disease triangle and influences both the pathogen and host. It is important that research methods represent conditions found in nurseries in order to assess disease progression accurately. Puddling observed in nurseries after heavy rains or over-irrigation and nursery practices that damage roots may increase susceptibility to pathogens.

Conclusion, objectives and statement of hypotheses

Phytophthora root rot is an important disease of containerized rhododendrons. Proper management of water and nutrition are important not only for rhododendron growth and flowering but are also important factors that impact disease. The overall goal of the research in this thesis was to examine the role of water, root damage, and N fertilizer application on Phytophthora root rot in rhododendrons.

The objective of experiment 1 (chapter 2) was to assess if research methods are realistic of disease development in nursery conditions, whether damage to roots increases disease severity, and whether these factors affect root rot caused by *P. cinnamomi* and *P. plurivora*. The experiment involved inoculating rhododendrons with *P. cinnamomi* and *P. plurivora* and then subjecting the plants to two water treatments and two damage treatments. The hypotheses tested were (1) Phytophthora root rot in plants inoculated with either *P. cinnamomi* or *P. plurivora* that have been flooded for 48 hours will occur more rapidly and disease will be more severe compared to nonflooded plants kept in saucers continuously filled with water, (2) Phytophthora root rot in plants inoculated with either *P. cinnamomi* or *P. plurivora* will occur more rapidly and be more severe in plants with high amounts of root damage than with less root damage, and (3) there will be no difference in disease between plants inoculated with *P. cinnamomi* and plants inoculated with *P. plurivora*. These results are expected because a flooded environment both promotes the inoculum level and activity of the pathogen and decreases host defense (Krebs 2013), because root damage both creates openings for pathogen entry and increases water stress (Coyier 1980), and because both *P.*

cinnamomi and *P. plurivora* are associated with severe root rot in rhododendrons (Weiland et al. 2018 in press).

The objective of experiment 2 (chapter 3) was to assess the impact of increasing N fertilizer rate on the progression and severity of Phytophthora root rot of rhododendron caused by *P. cinnamomi* and *P. plurivora*. The experiment involved inoculating rhododendrons with *P. cinnamomi* and *P. plurivora* and then subjecting the plants to three levels of incorporated slow release N. The hypotheses tested were (1) Greater rates of N fertilizer will cause more rapid disease progression and increase disease severity, and (2) There will be no difference in disease progression of disease severity between *P. cinnamomi* and *P. plurivora* at different rates of N fertilizer. These results are expected because high N alters host tissue to make it more easily penetrable by pathogens and because many *Phytophthora* species can assimilate N.

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Chapter 2 Is disease induced by flooding representative of nursery conditions in rhododendrons infected with *P. cinnamomi* or *P. plurivora*?

Abstract

Phytophthora root rot decreases availability and quality of rhododendrons produced in the USA. Symptoms of Phytophthora root rot include root necrosis, leaf chlorosis, stunting, and permanent wilt. *Phytophthora plurivora* and *P. cinnamomi* have been isolated from symptomatic stems and roots and frequently occur in surveys of Oregon nurseries and may play an important role in disease. Flooding for 24 to 48 h is commonly used in studies to induce disease, but flooding to the depths used in research studies rarely occurs in container nurseries. Instead, plants in containers may periodically sit in shallow pools of standing water in areas where drainage is poor or following excessive irrigation or heavy rain. Additionally, nursery production practices can damage roots. Root damage may play a role in predisposing plants to increased disease. Therefore, an experiment was conducted to determine whether disease induced by flooding in research studies is representative of disease progression under nursery conditions, whether damage to roots increases disease severity, and whether these affect root rot caused by *Phytophthora cinnamomi* and *P. plurivora*.

Rhododendron 'Boule de Neige' (trial 1) and 'Scintillation' (trials 2 and 3) with either low or high amounts of root damage were grown in a soilless substrate infested with *P. cinnamomi* and *P. plurivora*, then subjected to two water treatments for 18 weeks after inoculation (WAI) (trials 1 and 2) and 24 WAI (trial 3). Plants were either flooded for 48 h then maintained at or below container capacity or not flooded and kept in saucers of water to maintain substrate moisture ≥ 95 % container capacity. The flooded treatment

is typical of methods used to induce *Phytophthora* root rot in experimental conditions, while the plants in saucers were considered representative of nursery conditions when container plants sit in a shallow pool of water. Root rot and mortality were observed in both the flood and the saucer treatment. In general, root rot in plants inoculated with *P. cinnamomi* was more severe than in plants inoculated with *P. plurivora*. There were few differences in disease induced by flooding compared to disease induced by saucers in plants inoculated with *P. cinnamomi*. In contrast, there was more disease in plants inoculated with *P. plurivora* in the saucer treatment compared to the flood treatment. Across all pathogen and water treatments, there were no differences in disease between low and high physical root damage. *P. cinnamomi* inoculated plants had the lowest biomass and chlorophyll values, greatest frequency of wilt and death, and most severe root rot. Inoculating plants with *P. plurivora* caused little damage compared to controls. These results indicate that *P. cinnamomi* is generally a more aggressive pathogen than *P. plurivora* and suggest that disease induced by flooding may be representative of disease in container nurseries. These results indicate that it is not necessary to flood for 48 h to induce disease under experimental conditions. Future research should compare both the flood treatment and the saucer treatment to an inoculation method that maintains soil moisture around 60 to 70% container capacity, which is the recommendation for container rhododendrons, as high soil moisture content can change soil properties and alter host physiology. As rhododendrons require adequate drainage, high substrate moisture can result in water stress, physiological dysfunction, and root mortality.

Introduction

The 2012 value of greenhouse, nursery, and floriculture sales in the United States was \$14.5 B (USDA National Agricultural Statistics Service 2009). Of these sales, nursery stock (woody perennials) comprise 35.2%, a value of \$5.1 B. Oregon is ranked third in nursery stock production, accounting for 11% (\$500 M) of U.S. production (Oregon Department of Agriculture 2017). Nursery stock includes many types of trees and shrubs: deciduous shade trees, deciduous flowering trees, broadleaf evergreens, coniferous evergreens, and deciduous shrubs (USDA National Agricultural Statistics Service 2015). In the United States, 3,891 of the 8,226 (47%) nursery stock operations produce broadleaf evergreens, including species and cultivars of boxwood (*Buxus*), *Cotoneaster*, *Euonymus*, holly (*Ilex*), *Magnolia*, *Pieris*, *Pittosporum*, privet (*Ligustrum*), rhododendron and azalea (*Rhododendron*), and *Viburnum*. Of the 3,891 broadleaf evergreen operations, 761 (20%) produce rhododendrons, valued at over \$42 million. In Oregon, there were over 67 commercial nurseries that produced rhododendrons, accounting for over \$11 M in sales, the second highest state in rhododendron sales after Connecticut with over \$12 M (USDA National Agricultural Statistics Service 2009).

Phytophthora root rot decreases availability and quality of rhododendrons produced in the USA (Hoitink and Schmitthenner 1974). Symptoms of Phytophthora root rot include root necrosis, leaf chlorosis, stunting, and permanent wilt (Hoitink et al. 2014). One to two-year-old plants may develop a canker at the base of the stem as the pathogen moves from the roots to the stem. Phytophthora diseases of rhododendrons

can affect from 10% (Fraher 2014) to 100% (Weiland, personal communication; White 1937) of plants in a nursery. Multiple species of *Phytophthora* cause Phytophthora root rot, including *P. cinnamomi*, *P. cambivora*, *P. nicotianae*, *P. cryptogea*, and *P. citricola* (Hoitink et al. 2014). In a 2006 to 2009 survey of four Oregon nurseries, 28 *Phytophthora* taxa were identified from symptomatic and asymptomatic *Rhododendron*, *Pieris*, *Kalmia*, and *Viburnum* plant material (stems, roots, and leaves), potting material, soil, and water samples (Parke et al. 2014). The most commonly isolated species from plants were *P. plurivora* (33%) and *P. cinnamomi* (26%). Although multiple species cause Phytophthora root rot in rhododendron, most of the research has focused on *P. cinnamomi* (Benson and Parker 2005; Englander et al. 1980). There is comparatively little research on *P. plurivora*.

Studies show that high soil moisture increases disease severity for most host pathogen complexes (Blaker and MacDonald 1981; Krebs 2013; Sterne et al. 1977; White 1937). Severity of disease in different rhododendron cultivars inoculated with *P. cinnamomi* ranged from 35% to 90% higher in flooded conditions than nonflooded conditions (Krebs 2013). Severity of Phytophthora root rot in *Persea indica* inoculated with *P. cinnamomi* was highest between 0 kPa to -5 kPa (up to 90% diseased roots) and lowest at -10 kPa (10 to 50% diseased roots) (Sterne et al. 1977). While *P. cambivora* is able to cause disease in rhododendrons across a range of soil moisture (40-90% container capacity), symptoms appear more rapidly in high soil moisture (White 1937). In the absence of flooding, rhododendron 'Caroline' is resistant to *P. cinnamomi* (Blaker and MacDonald 1981). However, flooding for 24 to 48 h prior to

inoculation predisposed 'Caroline' to disease. *Rhododendron* 'Purple Splendor', on the other hand, showed no significant increase in disease due to flooding prior to inoculation.

Many studies of *Phytophthora* root rot commonly use a 48 h flood to stimulate root infection and then switch to a more standard irrigation regime after flooding is complete (Browne and Viveros 1999; Parke 2007; Weiland et al. 2010). In Browne and Viveros (1990) study almond trees were flooded for 48 h every 2 weeks starting 1 week after transplanting (at which point plants were up potted into containers with inoculum) for a total of 3 months (Browne and Viveros 1999). During flooding, potted plants were submerged so that there was 0.5 to 1 cm water above the soil surface and watered daily in between flooding events. In Parke's (2007) study, plants were flooded for 12 h every 7 to 9 days for a total of 6 weeks with an unspecified irrigation schedule between flooding events (Parke 2007). During flooding water was raised to a level of 2 cm below the soil surface. In Weiland et al's (2010) study, plants were flooded for 48 h with distilled water every 2 weeks starting 2 weeks after inoculation for 14 weeks (Weiland et al. 2010). During flooding water was maintained 0.5 cm above the soil surface and between flooding, plants were irrigated daily to field capacity.

Flooding to the depths used in research studies to increase zoospore production, rarely occurs in container nurseries. Instead, plants in containers may periodically sit in shallow pools of standing water in areas where drainage is poor or following excessive irrigation or heavy rain (Fig A 1). Studies have used saucers filled with water to represent standing water but have not compared the results to flooding (Tsao and

Garber 1960). It is unknown whether disease induced by flooding in research studies is representative of disease progression under nursery conditions.

Root damage can predispose plants to infection by *Phytophthora* as injury creates points of entry for the pathogen (Coyier 1980; White 1937). In addition, root damage can affect the plants ability to transport water, resulting in drought stress (Aldahadha et al. 2012) that may further predispose plants to infection (Blaker and MacDonald 1981). For example, rhododendron 'Caroline' became symptomatic between 21 and 35 days after inoculation with *P. cinnamomi* zoospores following a drought stress treatment in which water was withheld 8 to 10 days (Blaker and MacDonald 1981). In the absence of drought stress, the plants remained symptomless. Drought stressed plants had leaf water potentials of -16 to -20 bars compared to -2 to -6 bars in nonstressed plants, indicating a water deficit and change in root water absorption and leaf transpiration. It is unknown whether damage to roots that commonly occurs in nursery practices (during transplanting or root pruning) or as part of pathology research methods for plant inoculation increases disease severity.

Three trials were conducted to assess the influence of soil moisture and root damage on *Phytophthora* root rot. Rhododendron 'Boule de Neige' (trial 1) and 'Scintillation' (trials 2 and 3) with either low or high amounts of root damage were grown in a soilless substrate infested with *P. cinnamomi* and *P. plurivora*, then subjected to two water treatments for 18 weeks after inoculation (WAI) (trials 1 and 2) and 24 WAI (trial 3). Plants were either flooded for 48 h then maintained at or below container capacity or not flooded and kept in saucers of water to maintain substrate moisture \geq

95% container capacity. The plants in saucers were considered representative of conditions when nursery plants sit in a shallow puddle of water. The hypotheses tested were (1) Phytophthora root rot in plants inoculated with either *P. cinnamomi* or *P. plurivora* that have been flooded for 48 hours will occur more rapidly and disease will be more severe compared to nonflooded plants that were kept in saucers continuously filled with water, (2) Phytophthora root rot in plants inoculated with either *P. cinnamomi* or *P. plurivora* will occur more rapidly and be more severe in plants with high amount of root damage than with less root damage, and (3) there will be no difference in disease between plants inoculated with *P. cinnamomi* and plants inoculated with *P. plurivora*.

Materials and Methods

Experimental design

The experimental design was a full factorial, complete randomized block design with two pathogens (*P. cinnamomi* and *P. plurivora*) and a noninoculated control, two damage treatments (high and low), and two water treatments (flooded and saucer) replicated in 10 blocks. The experiment was conducted in a greenhouse in 2017 and repeated three times. Trials 1 and 2 ran for 18 weeks after inoculation (WAI). Trial 3 ran for 24 weeks WAI. Trial 1 inoculation was 22 February 2017. Trial 2 inoculation was 31 May 2017. Trial 3 inoculation was 14 August 2017. In addition to differences in season, cultivar, and age of plant, there were differences in irrigation, fertilization, and greenhouse conditions among trials (see below).

Isolate selection

P. cinnamomi and *P. plurivora* are commonly isolated from plant material and container substrate in Oregon nurseries (Parke et al. 2014). Isolate R056 (*P. cinnamomi*, GenBank Accession No. MG560189) and isolate R003 (*P. plurivora*, MG560192) were collected from Willamette Valley Oregon nurseries that grow rhododendrons in containers and had been used in previous pathogenicity experiments (Weiland et al. 2018). Isolates were identified based on morphology and from internal transcribed spacer (ITS) ribosomal DNA sequences matched at 99% to 100% identity to DNA sequences from type isolates of *P. cinnamomi* (KC478663 and FJ801806) and *P. plurivora* (FJ66522) (Jung and Burgess 2009; Scanu et al. 2013).

Inoculum preparation

Isolates of *P. cinnamomi* and *P. plurivora* were grown on PARP semi selective media for 2 weeks in a 20°C dark incubator. A mixture of V8 juice (Campbell Soup Company, Camden, NJ) and calcium carbonate (10 g CaCO₃/1000 ml V8) was filtered with 4 layers of cheese cloth to remove large particles, diluted with deionized water (160 ml V8/CaCO₃ and 1440 ml H₂O), and added to 3 liters of vermiculite (Therm-O-Rock-West Inc., Chandler, AZ). The V8/CaCO₃/vermiculite mixture was then placed in 15 liter plastic spawn bags (Fungi Perfecti, Olympia, WA) and autoclaved three times with 48 h between each run. Afterwards, a 2-week-old culture of each isolate was cut into 1.5 cm² squares, added to a separate bag of amended vermiculite, and maintained in a 20°C dark incubator. One plate of sterile PARP was used for a negative control treatment and added to a third bag of V8 amended vermiculite. After 1 week of incubation, all bags were mixed weekly to increase aeration and colonization. After 8 weeks, the infested

vermiculite was dried for approximately 3 days until friable and then stored in plastic bags in a 20°C dark incubator until plants were inoculated.

Inoculation method and damage treatments

The vermiculite inoculum was added to a soilless substrate (Metro-Mix 840PC, Sun Grow Horticulture, Agawam, MA) at a 10% v/v inoculum/substrate to achieve an inoculum level of 100 to 200 propagules per gram (ppg) of infested substrate. A slow release fertilizer (Harrell's 21-5-6 nursery polyon, Lakeland, FL) was also incorporated during inoculation at 3.56 kg/m³. The vermiculite inoculum, substrate, and fertilizer were mixed in a cement mixer for 10 minutes with a NaOCl wash between each pathogen. Noninfested vermiculite was used for the control.

Rhododendron cultivar 'Boule de Neige' (RHS #58) was used in trial 1 and cultivar 'Scintillation' (ARS 734) in trials 2 and 3. Both cultivars are reported as being susceptible to *P. cinnamomi* (Hoitink and Schmitthenner 1970; Hoitink and Schmitthenner 1975). Plants for trial 1 and 2 were obtained as rooted cuttings in L2 liner trays in June 2016 and repotted into standard 3.78 liter containers (black Poly-Tainer PT NS300 BFG, Supply Burton, OH) approximately 1.5 months later with soilless substrate (Metro-Mix 840PC, Sun Grow Horticulture, Agawam, MA) mixed with slow release fertilizer (Harrell's 21-5-6 Nursery polyon, Lakeland, FL) at 3.56 kg/m³. Plants were kept outdoors in a caryard, then brought into the greenhouse to acclimate approximately 2 months prior to inoculation. At time of inoculation plants were approximately 1.5 to 2 years old with an average total plant dry weight of 22 g for trial 1 and 38 g for trial 2. Plants for trial 3 were also obtained as rooted cuttings from the

same nursery, but were not repotted into larger containers prior to inoculation. Plants were approximately 8 months old at the beginning of the experiment with an average total plant dry weight of 5 g. Ten plants not used in experiments were sampled before each trial for root contamination by *Pythium* or *Phytophthora* and initial plant biomass data. *Pythium* species were isolated from roots of 40% of the plants at the beginning of trial 1 and *P. cinnamomi* was isolated from roots of 10% of plants in the beginning of trial 3. No *Pythium* or *Phytophthora* was detected from plant roots at the beginning of trial 2.

Plant root systems were subjected to low or high damage treatments before inoculation. For trial 3, as the plants were younger and smaller than the first two trials, a proportionately smaller amount of the root system was damaged. Low damage consisted of removing the bottom 5 cm of the root system in trials 1 and 2 or 2 cm of the root system in trial 3. High damage consisted of low damage plus four additional 2.5 cm deep cuts around the root system in trials 1 and 2 or four 1 cm deep cuts around the root system in trial 3. The removal of the lower 5 cm of root for trials 1 and 2 created space to place the infested or control substrate. Inoculum of each pathogen or control mix (800ml) was then placed into the bottom 5 cm of 2.5 liter polyethylene containers ((black Poly-Tainer PT NS300, BFG Supply Burton, OH). The plants were placed directly on top of the infested substrate in trials 1 and 2. For Trial 3, the plants were placed on a layer of noninfested substrate (approximately 4 cm) above the 5 cm layer of infested substrate at the bottom of the container.

Water treatments

Plants in both the flood and saucer treatments were watered every day with the same volume of water. Irrigation in both water treatments (flood and saucer) were similar throughout the experiment, except as follows. Two WAI, the substrate of plants in the flood treatment were saturated with water for 48 h by placing containers in a 3.78 liter bucket and irrigating until the water was 2.5 cm below the soil surface (Fig A 2). After 48 h the buckets were removed and the water was allowed to drain. Trial 3 plants in the flood treatment were flooded a second time 18 WAI. Immediately after inoculation, plants in the saucer treatment were placed in a saucer that was kept continuously filled with 1.5 cm of water, which maintained the plants $\geq 95\%$ container capacity.

The volumetric water content (VWC) of the substrate was monitored periodically (GS3 probe with ProCheck meter Decagon Devices, Inc., Pullman WA). Reader output was calibrated to substrate container capacity at the beginning of each experiment using the same substrate and containers used in the experiment. The volume and frequency of watering was adjusted to maintain a VWC at $\sim 75\%$ of container capacity (0.3 to $0.35 \text{ m}^3/\text{m}^3$) in the flood treatment and VWC $\sim 100\%$ container capacity ($>0.50 \text{ m}^3/\text{m}^3$) in the saucer treatment. In trial 2, the VWC of flooded plants during the 48 h flood was on average, $0.62 \text{ m}^3/\text{m}^3$ ($0.58 \text{ m}^3/\text{m}^3$ to $0.72 \text{ m}^3/\text{m}^3$; $>100\%$ container capacity). The VWC of flooded plants after the 48 h flood was on average in trials 1 to 3, respectively, $0.36 \text{ m}^3/\text{m}^3$ ($0.05 \text{ m}^3/\text{m}^3$ to $0.55 \text{ m}^3/\text{m}^3$; $\sim 79\%$ container capacity), $0.39 \text{ m}^3/\text{m}^3$ ($0.08 \text{ m}^3/\text{m}^3$ to $0.72 \text{ m}^3/\text{m}^3$; $\sim 83\%$ container capacity), $0.40 \text{ m}^3/\text{m}^3$ ($0.10 \text{ m}^3/\text{m}^3$ to $0.51 \text{ m}^3/\text{m}^3$; $\sim 84\%$ container capacity). The VWC of plants in saucers was on average in

trials 1 to 3, respectively, 0.48 m³/m³ (0.36 m³/m³ to 0.58 m³/m³; 95% container capacity), 0.51 m³/m³ (0.32 m³/m³ to 0.60 m³/m³; 98% container capacity), 0.54 m³/m³ (-0.01 m³/m³ to 0.63 m³/m³; >100% container capacity) (Table A 1).

Greenhouse conditions

Greenhouse temperature was set to approximately 15°C night and 18°C day. There was 16 h of supplemental light provided daily (LumiGrow 325, Lumigrow Emeryville, CA), starting 5 days after inoculation. Blue (18% output), white (13% output) and red (69% output) LEDs were set at full intensity. The total photon flux capacity was 530 µmol/sec. There was approximately 4 lights/m² above greenhouse benches. Environmental sensors measured air temperature (T_{air}), relative humidity (RH) (Vaisala HMP60, Vaisala Co., Helsinki, Finland) and photosynthetically active radiation (PAR) (Licor Quantum, LI-COR Inc., Lincoln, NE) and the data were logged hourly (LI 1400, LI-COR Inc., Lincoln, NE).

Daily average PAR in trials 1 to 3, respectively, was 325 µmol s⁻¹ m⁻² (1828 µmol s⁻¹ m⁻² max), 240 µmol s⁻¹ m⁻² (852 µmol s⁻¹ m⁻² max), and 438 µmol s⁻¹ m⁻² (2139 µmol s⁻¹ m⁻² max) (data now shown). Daily average T_{air} in trials 1 to 3, respectively was 19°C (13°C min, 30°C max), 21°C (16°C min, 46°C max), and 20°C (16°C min, 30°C max) (Fig A 3). Daily average RH in trial 1-3, respectively was 51% (17% min, 85 % max), 62% (29% min, 80% max), and 47% (17% min, 93% max) (data not shown).

Leaf color was used to evaluate plant nutrient status and schedule fertilizer application. Color (relative units of chlorophyll, anthocyanin, and flavonol) of current season leaves was monitored weekly on three fully expanded leaves per plant using a

Dualex Scientific sensor (Force-A, Orsay Cedex, France). When values measured on leaves of noninoculated plants trended below 30 relative chlorophyll units, all plants were fertilized with a water soluble fertilizer in lieu of water at 0.62 g N/3.78 L (MiracleGrow Azalea, Camellia, Rhododendron Plant Food 30-10-10, Miracle-Grow Inc., Marysville, OH).

Disease and plant health evaluation

Visual symptoms of disease (presence or absence of wilt and mortality) and leaf color (see above) were recorded weekly for each plant. At the end of each trial, shoots were removed from plants and separated into leaves and stems, and the substrate was washed from roots. In trials 1 and 2, roots were divided between core roots (original root system) and outside-core roots (all roots except original root system). In trial 3, the root system was left intact. A 1 cm stem sample and four 1 cm root samples from random locations around the root system were taken from each plant and plated on PARP for pathogen isolation. Root system health was rated on a scale of 1 to 3 where 1 = healthy with >50% of root system with white, healthy roots, 2 = moderate root rot with < 50% of root system with white, healthy roots, and 3 = severe root rot with >75% of root system with dark, dead roots. Stem, leaves, and roots were dried at 60°C in a forced air oven (VWR Scientific Products manufactured by Sheldon Manufacturing, Inc., Cornelius, OR) until a constant weight was achieved then dry weight for each plant part was measured.

Statistical analysis

All data was analyzed using Statistica, version 13 (TIBCO Software Inc., Palo Alto, CA). Frequency data (visual symptoms, root health ratings, and pathogen

isolation) were analyzed using generalized linear models. For each week, a binomial distribution with logit link function was used for analyzing wilt, mortality, and pathogen isolation data and an ordinal multinomial distribution with logit link function was used for the analysis of root rot data. Models for generalized linear analyses included block, trial, pathogen treatment, water treatment, and damage treatment as main effects in a full factorial design. Forward and backward stepwise selection was used for model selection and differences among treatments evaluated using Yates corrected Chi square, (Mantel 1974) and Fisher's exact test (Agresti 1992) at $P < 0.05$. Data are presented as the percentage (%) of plants in each category.

For all other data, normality was assessed by examining normal P-P plots (Neter et al. 1985) and equal variance was assessed by Levene's test for homogeneity of variances (Glass and Hopkins 1996). Weekly leaf color data were analyzed using repeated measures ANOVA with block in the model and pathogen, water treatment, damage treatment, and WAI as main effects in a full factorial design. Interactions between main effects and time were verified using Pillai's, Hotelling's, and Roy's multivariate tests (Finn 1974). Trial 1 chlorophyll data was log transformed and trial 2 chlorophyll data was squared to pass Levene's test for homogeneity of variance prior to ANOVA. ANOVA models used full factorial designs of pathogen, water treatment, and damage treatment as main effects and block was included as a random effect. Root core biomass (trial 3), leaf biomass (trial 2), and stem biomass data (all trials) was assessed using ANOVA. Stem biomass (all trials) was log transformed prior to ANOVA to pass Levene's test for homogeneity of variance. Means of significant effects from

ANOVA were evaluated using Tukey's HSD post hoc test at $P < 0.05$. When data required transformation, untransformed data are presented. Total plant biomass (all trials), total root biomass (trial 1), core root biomass (trial 2), outside core root biomass (trials 2 and 3), and leaf biomass (trials 1 and 3), were analyzed using the Kruskal Wallis ANOVA and Median Test, and differences among means were assessed at $P < 0.05$ (Siegel and Castellan 1988).

Results

Noninoculated controls

Noninoculated control plants were generally larger and as green as, or greener than other treatments. Plant biomass varied among trials and plants in trial 3 were smaller because they were younger than plants in trials 1 and 2 (Fig. 1). In general, plants in the saucer treatment had lower biomass than plants in the flood treatment. Plants in the saucer treatment had smaller root biomass outside of the core of the root system (trials 2 and 3) and smaller stem and leaf biomass (trial 3 only) compared to the flood treatment ($P \leq 0.001$) (Fig. 1). Chlorophyll values varied among trials, and were generally lowest in trial 3 (Fig. 2 A,C,E). Compared to the flood treatment, plants in the saucer treatment also had periodically lower chlorophyll values in trials 1 (9 WAI) and 3 (15 WAI), and periodically lower or higher chlorophyll values in trial 2 ($P \leq 0.001$) (Fig. 2 B, D, F). Anthocyanin values were similar or greater in the saucer treatment than the flood treatment in trials 1 and 3 and similar or lower than values in the flood treatment in trial 2 ($P = 0.003$) (Fig. 3). Leaf flavonol values of control plants were not influenced by water treatment ($P > 0.063$). Few control plants had symptoms of disease even though

some plants were contaminated with pathogens (Table 1). Across all three trials, only five plants wilted and only one plant died in trial 3 (Fig. 4 and Fig. 5).

In trials 1 and 2, the roots of the majority of control plants were healthy or had moderate root rot (Fig. 6A and Fig. 6B). In contrast, trial 3 control plants had relatively equal numbers of root systems that were either healthy, or had moderate or severe root rot (Fig. 6C). Across all trials, most of the control plants with moderate or severe root rot were in the saucer treatment ($P \leq 0.001$) (Fig. 7). Four of the five plants that wilted were in the saucer treatment, but neither *Pythium* nor *Phytophthora* were isolated from any of the roots. The control plant that died was also in the saucer treatment, and both *Pythium* and *P. cinnamomi* were isolated from the roots of the dead plant (Table 1). Root contamination by *Pythium* occurred in all three trials and ranged from 40% (trial 1) to 85% (trial 3) of all control plants (Table 1). Root contamination by *P. cinnamomi* occurred in the roots of 5% (2/40) of the control plants (Table 1), but not in trial 1 or 2 (data not shown). Water treatment had no effect on any other of the measurements in control plants and damage treatment had no effect on any of the measured variables in control plants ($P > 0.050$).

P. plurivora

Compared to noninoculated controls, plants inoculated with *P. plurivora* were periodically less healthy. Plants inoculated with *P. plurivora* had similar plant biomass as controls in all trials (Fig. 1), similar leaf anthocyanin and flavonol values in all three trials, but lower chlorophyll values in trial 2 (Fig. 2 C, Fig. 3, flavonol values not shown). Water treatments had little influence on leaf color of controls and *P. plurivora* plants,

except for in trial 1. In trial 1, plants inoculated with *P. plurivora* had higher leaf anthocyanin values in the saucer treatment than the flood treatment, but were generally similar to those observed in the controls in both treatments (Fig. 3A and B). There was more wilting in *P. plurivora* inoculated plants than controls. By the end of trials 1, 2, and 3, respectively, 40%, 20%, and 10% of plants inoculated with *P. plurivora* wilted (Fig. 4). By the end of trial 1, there was also greater mortality in plants inoculated with *P. plurivora* than in controls (Fig. 5). In contrast, there was little to no mortality in *P. plurivora* inoculated plants in trials 2 or 3, which was similar to what was observed in the controls.

In trial 1, there were fewer *P. plurivora* inoculated plants with healthy roots than in the control treatment. However, in trials 2 and 3, the percentage of plants with healthy roots or moderate root rot was the same as that observed in the controls (Fig. 6). Flooded control and *P. plurivora* plants generally had more healthy root systems and fewer root systems with moderate root rot than those in saucers. Control plants had more healthy root systems and less severe root rot than *P. plurivora* plants in the flood treatment. Control plants had less moderate root rot than *P. plurivora* plants in the saucer treatment (Fig. 7). Root biomass of plants in the saucer treatment and flood treatment were similar to controls in all trials (Fig. 1). Root isolation of *P. plurivora* differed among trials. Generally, isolation was similar across tissue types (roots vs. stems), water treatments (flood vs. saucer), and damage treatments (low vs. high) except in trial 1, where *P. plurivora* was more frequently isolated in the saucer treatment than the flood treatment ($P = 0.011$). However, isolation of *P. plurivora* increased with

increasing root rot (Table 2). Water treatment had no influence on any other of the measurements in plants inoculated with *P. plurivora* and there were no effects of damage treatment on plants inoculated with *P. plurivora* ($P > 0.050$).

Root contamination by *Pythium* was observed on 28% (trials 1 and 2) to 60% (trial 3) of all inoculated plants and by *P. cinnamomi* on the roots of 10% of the inoculated plants in trial 3 (Table 1). There was no difference in the frequency of *Pythium* isolations or *P. cinnamomi* isolations between water treatments of *P. plurivora* inoculated plants ($P > 0.369$).

P. cinnamomi

Compared to noninoculated controls and those inoculated with *P. plurivora*, plants inoculated with *P. cinnamomi* were often less healthy. Total plant biomass of *P. cinnamomi* plants was lower than controls and *P. plurivora* plants in trials 1 and 2 and similar to controls and *P. plurivora* plants in trial 3 (Fig. 1). Leaf chlorophyll values of *P. cinnamomi* plants were lower than controls and *P. plurivora* plants in trial 1, lower than controls but similar to *P. plurivora* plants in trial 2, and similar to controls and *P. plurivora* plants in trial 3 (Fig. 2 A, C, E). Chlorophyll values were lower than controls and *P. plurivora* plants starting 9 WAI in trial 1, but only lower than controls starting 16 WAI in trial 2. Water treatments had similar effects on leaf chlorophyll or flavonol values in all pathogen treatments. Leaf anthocyanin values of *P. cinnamomi* plants between water treatments did not differ in trials 2 and 3 and were generally similar to those observed in control plants (Fig. 4). Leaf flavonol values of *P. cinnamomi* plants were not influenced by water treatment ($P > 0.063$, data not shown).

The majority of *P. cinnamomi* plants wilted and approximately half died during trials 1 and 2 (Fig. 4 and Fig. 5). Compared to controls and *P. plurivora* plants, more *P. cinnamomi* plants wilted by 7 WAI in trials 1 and 2 and more were dead by 10 WAI in trial 1 and 8 WAI in trial 2. Few *P. cinnamomi* plants wilted and died in trial 3, where there was no difference in wilt or mortality compared to the controls or *P. plurivora* plants.

The majority of *P. cinnamomi* plants had more moderate to severe root rot compared to controls and *P. plurivora* plants (Fig. 6 and Fig. 7). In trials 1 and 2, >95% of *P. cinnamomi* plants had severe root rot compared to <2% of control plants and < 13% of plants inoculated with *P. plurivora*. In trial 3, both *P. cinnamomi* and *P. plurivora* plants more frequently had moderate root rot and less frequently had healthy root systems than controls. In contrast to *P. plurivora* isolations, *P. cinnamomi* was isolated from the majority of roots in trials 1 and 2, regardless of root rot severity (Table 2). In all trials, there was no difference in the frequency of *P. cinnamomi* isolations between flood or saucer treatments. In contrast more *P. plurivora* was isolated from the saucer treatment than the flood treatment, but only in trial 1.

Pythium was isolated less frequently from *P. cinnamomi* plants than from control plants and *P. plurivora* plants in trials 1 and 2, and as frequently from *P. plurivora* plants, but less frequently than control plants, in trial 3. *Pythium* was observed to contaminate 8% (trials 1 and 2) to 63% (trial 3) of all roots in plants inoculated with *P. cinnamomi* (Table 1). Water treatment had no influence on any other of the

measurements in plants inoculated with *P. cinnamomi* and there were no effects of damage treatment on plants inoculated with *P. cinnamomi* ($P > 0.050$).

Discussion

High substrate moisture for prolonged time decreased health of rhododendrons when plants were not inoculated with *Phytophthora*. Control plants in saucers where substrate remained $\geq 95\%$ container capacity had greater root rot in trials 2 and 3 compared to control plants that were flooded, then subsequently maintained at $\sim 80\%$ container capacity. As rhododendrons require adequate drainage, high substrate moisture can result in water stress, physiological dysfunction, and root mortality (Kozlowski 1997). Root damage may be due to: 1) anoxia 2) increased host susceptibility to opportunistic microorganisms, 3) increased inoculum and activity of said microorganisms or more likely 4) a combination of these factors. In the present study, the plants in saucers were maintained at higher substrate moisture than those in the flood treatment for ≥ 18 weeks while plants in the flood treatment were only maintained at greater substrate moisture than the saucer treatment for 48 h. We therefore suspect that the roots of control plants in saucers were under more water stress than those in the flood treatment. The longer duration of water stress in the saucer treatment may have compromised plant health due to a combination of anoxia and the presence of *Pythium*, which we detected on the roots of plants at the beginning of the study.

In two trials, *Pythium* was more frequently isolated from control plants in saucers than flooded control plants, suggesting that it was a contributing factor to decreased root health. For example, *Pythium cryptoirregulare* has previously been shown to

periodically decrease rhododendron plant health (Weiland et al. 2018 in press). To confirm whether the damage of control plants was due to the *Pythium* species in this study, pathogenicity studies would need to be conducted using the *Pythium* isolates obtained during the study on the same rhododendron cultivars. An earlier study of Phytophthora root rot on citrus also used saucers continuously filled with water to create an environment conducive to disease but found that root damage occurred due to anoxia, not specifically to presence of a pathogen (Tsao and Garber 1960). In comparison to regular watering and continuous water logging, in which the saucers were always filled with water, only periodic water logging, allowed for both adequate root growth of noninfested plants and progression of rot in infested plants. Tsao and Garber's study emphasized an important criterion for optimization of inoculation methods: a watering regime that is conducive to both root growth and disease.

Regardless of water treatment, plants inoculated with *P. cinnamomi* had the lowest biomass and chlorophyll values, greatest frequency of wilt and death, and most severe root rot compared to all other treatments. The high level of root rot in trials 1 and 2 in plants inoculated with *P. cinnamomi* was expected. Both the seed and pollen parent of 'Boule de Neige' and the seed parent of 'Scintillation' are in the subgenus *Hymenanthes*. There is less than 3% frequency of resistance to *P. cinnamomi* in this subgenus and the inheritability of resistance is low (Krebs 2013). *Phytophthora cinnamomi* is one of the most damaging pathogens of rhododendrons and these results support this designation (Beaulieu et al. 2017). Even though the water treatments were different enough from each other to affect biomass, leaf color and root quality of

controls, they may not have been different enough from each other to affect disease in plants inoculated with *P. cinnamomi*. Substrate in the saucer treatment, and in the flood treatment, both during the 48 h period of saturation and after, had water contents above 75% container capacity. Sporangia and zoospores are observed at 50% water content (vol. H₂O/w dried soil) (Reeves 1975). The level of inoculum and activity of the pathogen was likely great enough in both water treatments to cause similar root damage.

Inoculating plants with *P. plurivora* caused little damage compared to controls or to plants inoculated with *P. cinnamomi*. *P. plurivora* was expected to cause more disease because previous studies showed that *P. plurivora* was associated with, or caused severe root rot in rhododendrons (Lilja et al. 2011; Schoebel et al. 2014; Weiland et al. 2018 in press). However, the cultivars that were used in the previous studies were not used in this present experiment, and differences in cultivar susceptibility may account for the low disease in plants inoculated with *P. plurivora* in our study. Previous research has shown that both ‘Boule de Neige’ and ‘Scintillation’ are susceptible to *P. cinnamomi*, but these cultivars have not previously been evaluated with *P. plurivora* and might not be as susceptible to this pathogen. Of the 120 plants inoculated with *P. plurivora* in the present study, 13% ‘Boule de Neige’ and 1% ‘Scintillation’ plants died. In Weiland’s 2018 study, mortality of plants inoculated with *P. plurivora* ranged from 65% to 50% for Rhododendron ‘Cunningham’s White’ and from 57% to 20% for ‘Yaku Princess’ (Weiland et al. 2018). Our results also indicate that cultivars that are susceptible to *P. cinnamomi* may not be susceptible to *P. plurivora*.

This is in contrast to results that indicated that the two cultivars evaluated were susceptible to both *P. cinnamomi* and *P. plurivora* (Weiland et al. 2018 in press).

Low disease in plants inoculated with *P. plurivora* also may be explained by differences of the effect of moisture on the biology of the organism and on disease. The effect of moisture on different *Phytophthora* species and on disease is variable. In vitro studies show that the effects of moisture on mycelia growth, chlamydospore lysis, and sporangia and oospore production varies by species (Hardy and Sivasithamparam 1991; Reeves 1975; Sommers et al. 1970). While *P. megasperma* and *P. parasitica* had optimal growth at matric potentials below -1000 kPa, *P. cinnamomi* had optimum growth between -1000 kPa and -1500 kPa (Sommers et al. 1970). The range of media matric potentials in which propagules of *P. citricola* are produced is broader than that of *P. cinnamomi* (Hardy and Sivasithamparam 1991). *P. cinnamomi* produced sporangia from -2.5 kPa to -10.0 kPa, with highest production at -10 kPa and had no chlamydospore lysis between -2.5 kPa and -10 kPa (for reference 0 kPa = saturation). In soil, *P. cinnamomi* produced sporangia and zoospores at 50% water content (vol H₂O/w dried soil) and produced chlamydospores from 5 to 50% water content (Reeves 1975). *P. citricola* produced sporangia between matric potentials of -1 kPa to -10 kPa, with highest production between -2.5 kPa to -7.5 kPa and produced oospores between -1 kPa to -10 kPa, with highest production at -10 kPa (Hardy and Sivasithamparam 1991). It may be that certain isolates of *P. plurivora* do not act similar to *P. citricola* and that the water content in the flood and saucer treatments were not within the optimal range for sporangia production. Alternatively, *P. plurivora* may be less virulent than *P. cinnamomi*.

In general, despite differences in cultivars between trials 1 and 2, disease was much more severe than in trial 3. It was expected that trial 3 plants inoculated with *P. cinnamomi* or *P. plurivora* would have more disease than trial 1 and 2 because the plants were younger, and juvenile plants, with more succulent tissue and limited root systems, are generally thought to be more susceptible to Phytophthora root rot than more mature, woodier plants (Coyier 1980). Our analysis of leaf color suggest that plant nutrition may have influenced disease severity. Chlorophyll values at 5 WAI in plants inoculated with *P. cinnamomi* were much lower in trial 3 (22 relative units) than in trials 1 (32 units) and 2 (36 units) where root rot was much more severe in plants inoculated with *P. cinnamomi*. Leaf greenness (relative chlorophyll units) are corrected with plant nitrogen (N) status. Low plant N status in trial 3, in comparison with trials 1 and 2, may explain the low amount of disease in plants inoculated with *P. cinnamomi*. Phytophthora dieback was more severe in rhododendrons with high nitrogen concentration in young foliage (Hoitink et al. 1986). Future research should address the impact of low and high N on disease.

Low disease in trial 3 could also be a result of differences in inoculum placement among trials. In trial 3, because of the small root size, there was a noninfested soilless substrate between the root system and inoculum. In trials 1 and 2, the root system was in direct contact with the inoculum.

There is no literature specifically on the effect of root damage on Phytophthora root rot in rhododendrons. In our study, there were no differences in disease between plants in the low and high damage treatment. *P. cinnamomi* was able to cause severe

root rot and mortality even at the low damage treatment and high root damage did not lead to additional mortality in plants inoculated with *P. plurivora*. Little difference in disease between the damage treatments may be due to the damage treatments being too similar to each other. Alternatively, the inoculum density and the aggressiveness of the pathogen may have been high enough to overwhelm any differences between the two damage treatments. To further assess whether root damage prior to inoculation predisposes plants to infection, the experiment could be repeated with the addition of a non-damage treatment.

Results fail to support all three hypotheses tested. Results show that (1) Phytophthora root rot in plants inoculated with either *P. cinnamomi* or *P. plurivora* that have been flooded for 48 h did not occur more rapidly and disease was not more severe compared to nonflooded plants that were kept in saucers continuously filled with water. (2) Phytophthora root rot in plants inoculated with either *P. cinnamomi* or *P. plurivora* did not occur more rapidly and were not more severe in plants with high amount of root damage than with less root damage. (3) There were differences in disease between plants inoculated with *P. cinnamomi* and plants inoculated with *P. plurivora*.

These results suggest that disease induced by flooding is representative of disease in container nurseries when plants sit in a shallow pool of water. These results also show that it is not necessary to flood for 48 h to induce disease under experimental conditions. Root rot and mortality were also observed in the saucer treatment where soil was maintained $\geq 95\%$ container capacity for an extended period of time. Because root rot damage occurred in the noninoculated plants of the saucer treatment, future

experiments should continue to use the flood treatment to induce disease. Future research should also compare both the flood treatment and the saucer treatment to an inoculation method that maintains soil moisture around 60 to 70% container capacity, as a high soil moisture content can change soil properties and alter host physiology (Kozlowski 1997).

Figures and Tables

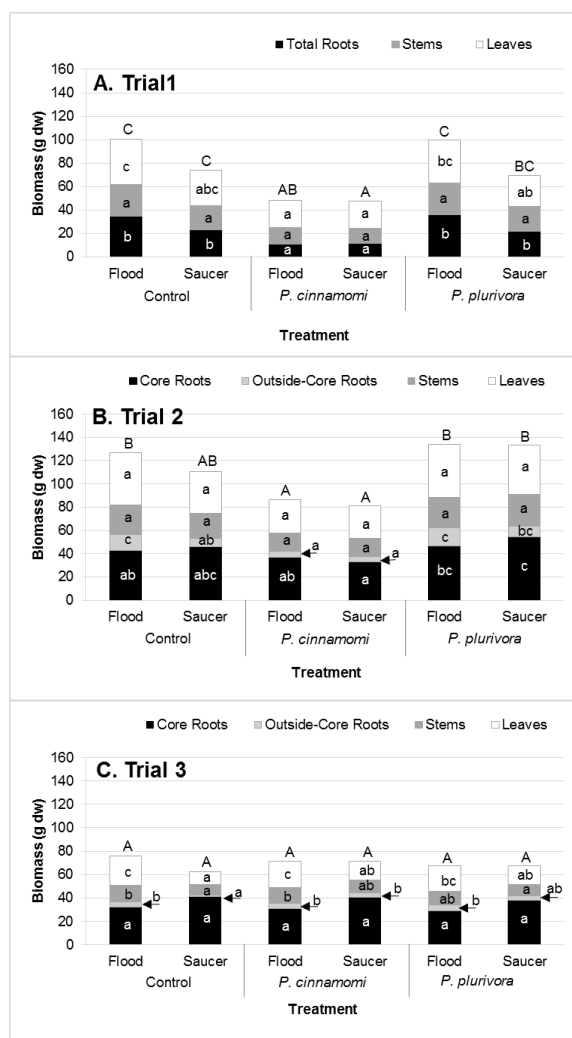


Fig. 1 Biomass of rhododendron plants' (Rhododendron 'Boule de Neige' trial 1 and 'Scintillation' trials 2 and 3) either not inoculated (Control) or inoculated with *Phytophthora cinnamomi* or *P. plurivora* and grown in two different water treatments (Flood and Saucer). Biomass measured 18 weeks after inoculation (WAI) for trial 1 (A) and 2 (B) and at 24 WAI for trial 3 (C). Total biomass is the sum of biomass in roots, stems, and leaves. Flood: substrate saturated for 48 h 2 WAI for all trials and at 18 WAI for trial 3. Saucer: containers were placed in a saucer that was continuously filled with 1.5 centimeters of water. Plants in both the flood and saucer treatments were watered every other day with the same volume of water. Values are means across damage treatments (n=20). Values within a tissue with different lower case letters across treatments or upper case letters for total biomass across treatments are significantly different at $P \leq 0.05$.

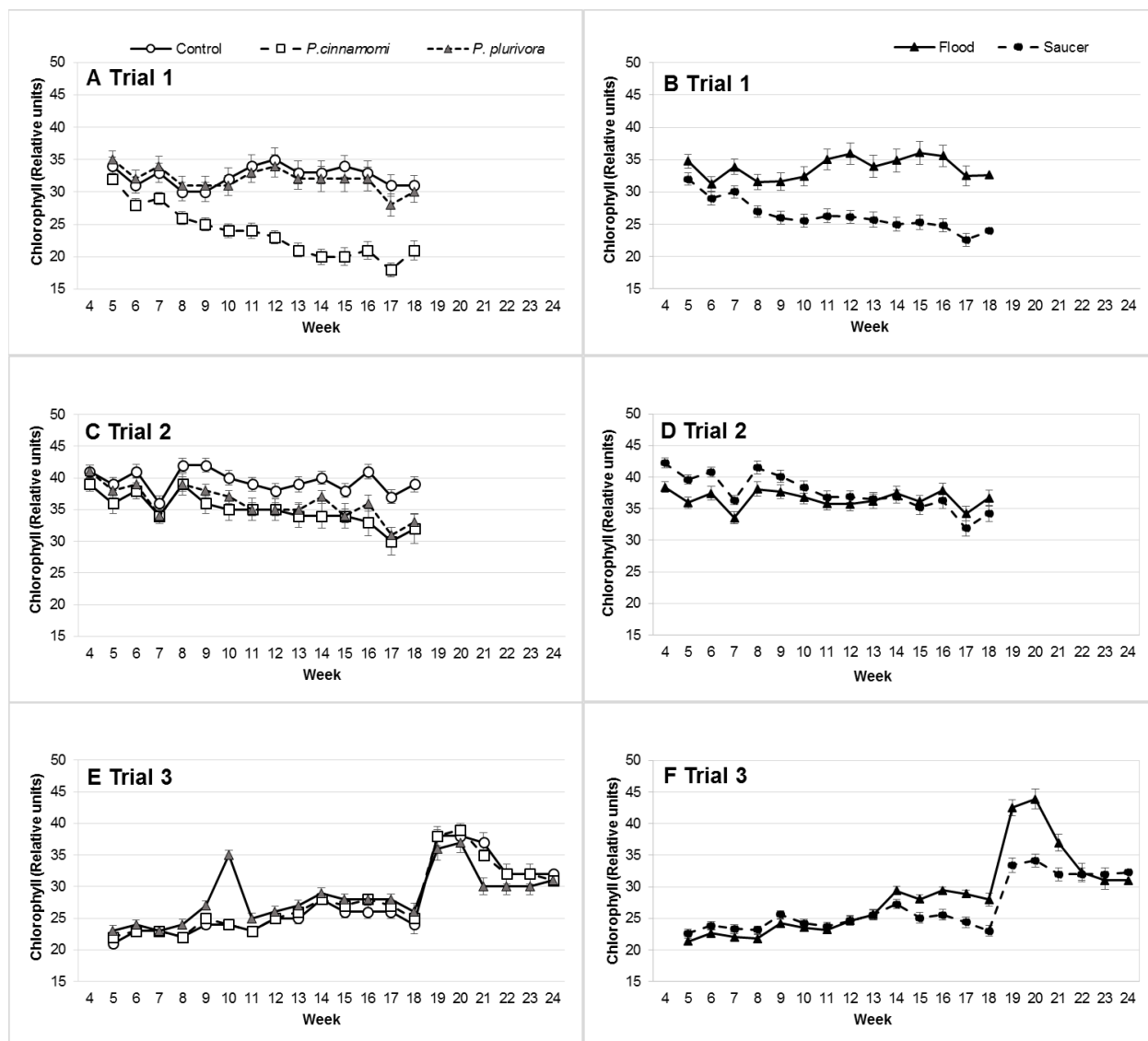


Fig. 2 Chlorophyll values from leaves on Rhododendron plants (Rhododendron 'Boule de Neige' trial 1 and 'Scintillation' trials 2 and 3) either not inoculated (Control) or inoculated with *Phytophthora cinnamomi* or *P. plurivora* in trial 1 (A), 2 (C), and 3 (E) and grown with two different water treatments (Flood, Saucer) in trials 1 (B), 2 (D), and 3 (F). Flood: substrate saturated for 48 h 2 WAI and at 18 WAI in trial 3. Saucer: containers were placed in a saucer that was continuously filled with 1.5 centimeters of water. Plants in both the flood and saucer treatments were watered every other day with the same volume of water. (A, C, E) Values are means across damage and water treatments (n=40) and error bars show mean standard error. (B, D, F) Values are means across pathogen and damage treatments (n=60) and error bars show mean standard error.

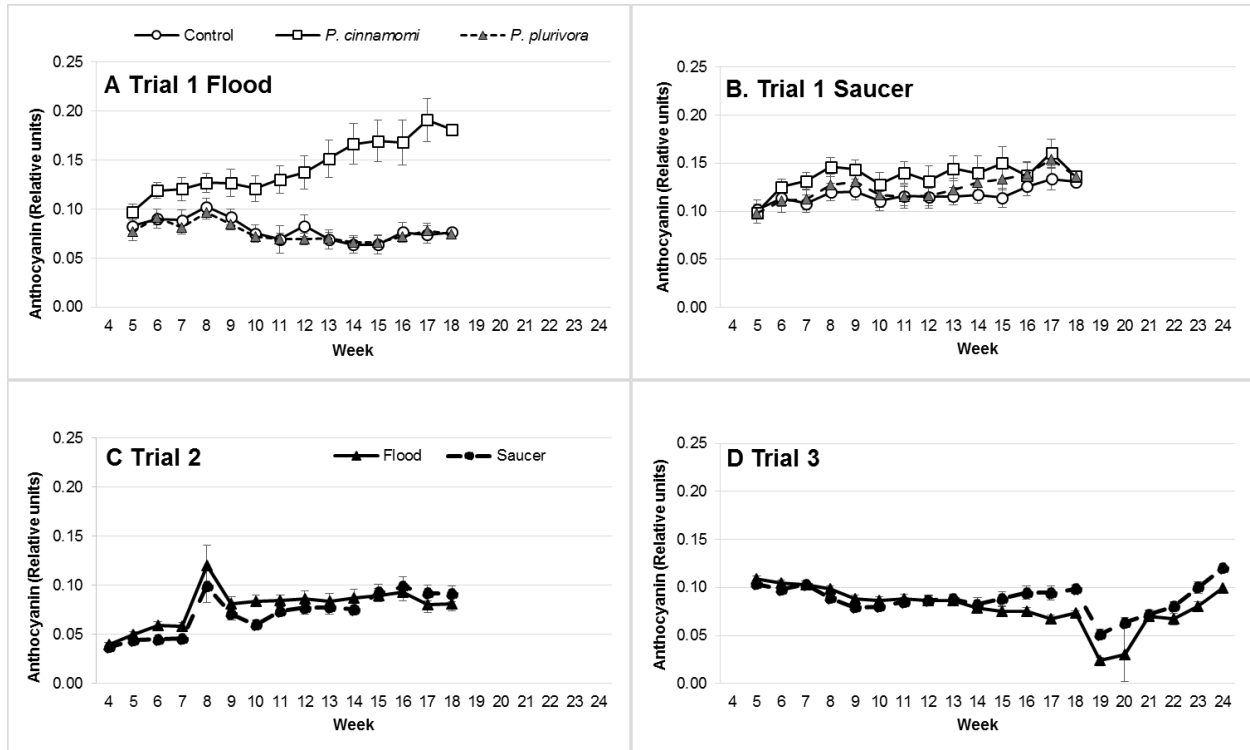


Fig. 3 Anthocyanin values from leaves on rhododendron plants (Rhododendron 'Boule de Neige' trial 1 and 'Scintillation' trials 2 and 3) either not inoculated (Control) or inoculated with *Phytophthora cinnamomi* or *P. plurivora* and grown in two different water treatments (Flood, Saucer) in trial 1 (A and B) and averaged across pathogens in trial 2 (C) and 3 (D). Flood: substrate saturated for 48 h 2 WAI for all trials and at 18 WAI for trial 3. Saucer: containers were placed in a saucer that was continuously filled with 1.5 centimeters of water. Plants in both the flood and saucer treatments were watered every other day with the same volume of water. Values are means across damage treatment in trial 1 (A and B; n=20) and pathogen and damage treatments in trial 2 (C) and 3 (D) (n=60). Error bars show mean standard error.

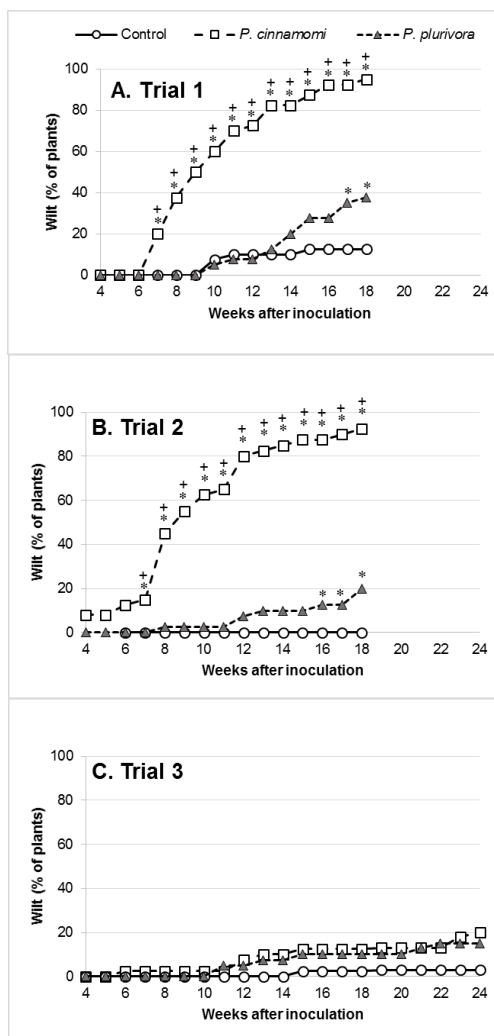


Fig. 4 Wilt in rhododendron plants (Rhododendron 'Boule de Neige' trial 1 and 'Scintillation' trials 2 and 3) either not inoculated (Control) or inoculated with *Phytophthora cinnamomi* or *P. plurivora* in trials 1 (A), 2 (B) and 3 (C). Values are means across all water and damage treatments. Significant differences of each pathogen from controls (*) or from each other (+) are indicated at $P \leq 0.050$.

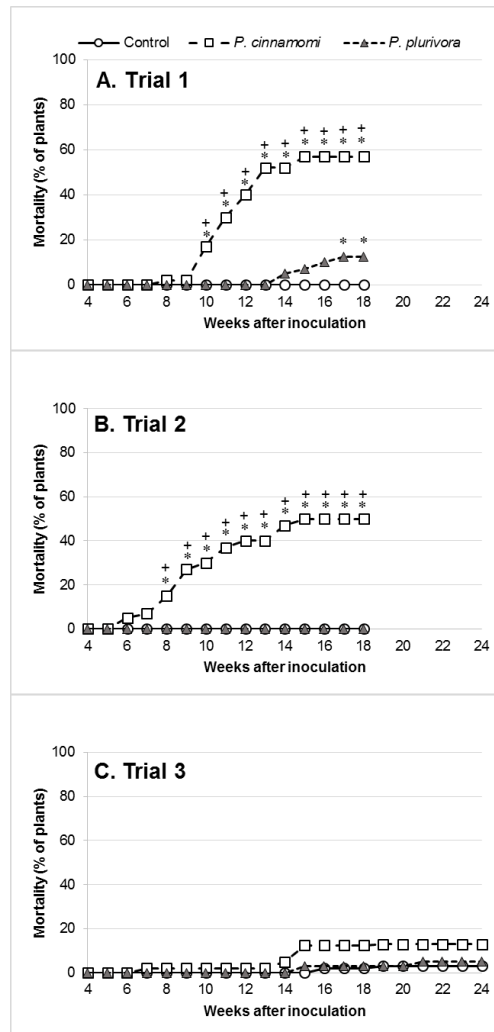


Fig. 5 Mortality in rhododendron plants (Rhododendron ‘Boule de Neige’ trial 1 and ‘Scintillation’ trials 2 and 3) either not inoculated (Control) or inoculated with *Phytophthora cinnamomi* or *P. plurivora* in trials 1 (A), 2 (B) and 3 (C). Data points are means across all water and damage treatments. Significant differences of each pathogen from controls (*) or from each other (+) are indicated at $P \leq 0.050$.

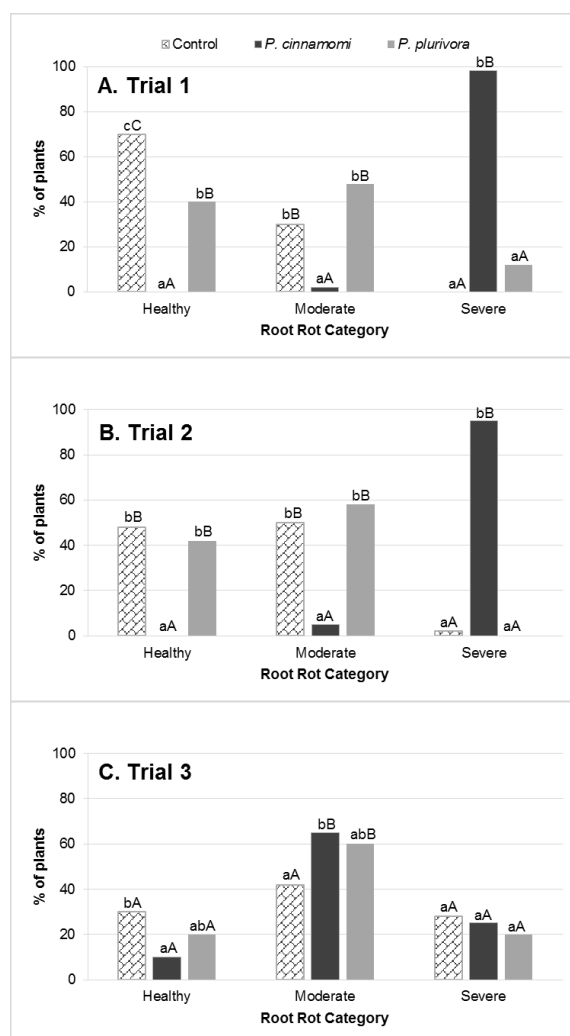


Fig. 6 Root rot in rhododendron plants (Rhododendron 'Boule de Neige' trial 1 and 'Scintillation' trials 2 and 3) either not inoculated (Control) or inoculated with *Phytophthora cinnamomi* or *P. plurivora* in trial 1 (A), 2 (B) and 3 (C). Root rot was evaluated at 18 weeks after inoculation (WAI) for trials 1 and 2 and 24 WAI for trial 3. Healthy (>50% of root system with white healthy roots), moderate root rot (<50% of root system with white healthy roots), and severe root rot (>75% of root system with dark, dead roots). Values are means across damage treatments and water treatments (n=40). Values with different lower case letters within a root rot category or upper case letters among root rot categories are significantly different at $P \leq 0.05$.

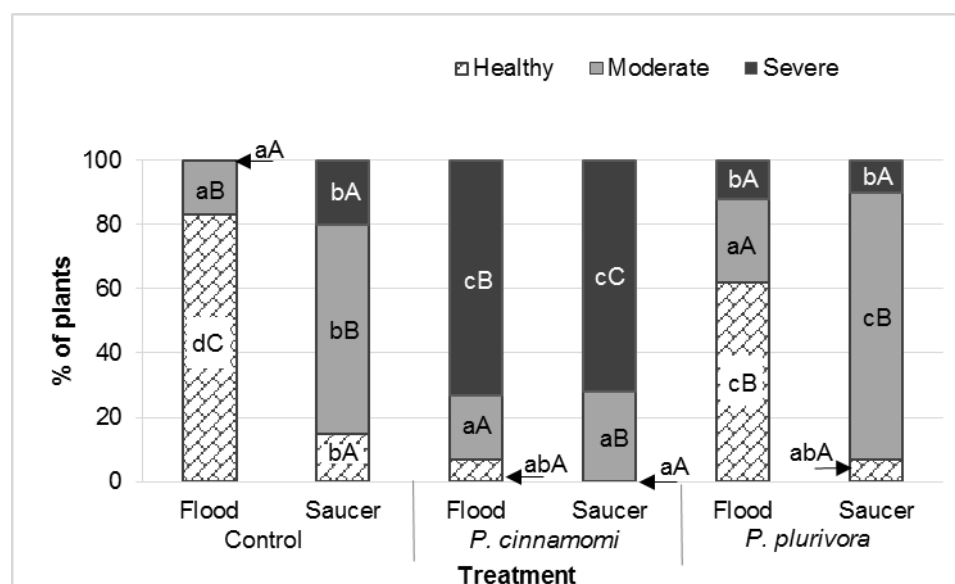


Fig. 7 Root rot in rhododendron plants (Rhododendron 'Boule de Neige' trial 1 and 'Scintillation' trials 2 and 3) either not inoculated (Control) or inoculated with *Phytophthora cinnamomi* or *P. plurivora* and grown with two different water treatments (Flood, Saucer). Flood: substrate saturated for 48 h 2 WAI for all trials and at 18 WAI for trial 3. Saucer: containers were placed in a saucer that was continuously filled with 1.5 centimeters of water. Plants in both the flood and saucer treatments were watered every other day with the same volume of water. Root rot was evaluated at 18 WAI for trial 1 and 2 and 24 WAI for trial 3. Healthy: (>50% of root system with white healthy roots), moderate root rot (<50% of root system with white healthy roots), and severe root rot (>75% of root system with dark, dead roots). Values are means across all trials and damage treatments (n=60). Values with different lower case letters among pathogen water treatments within a root rot category and upper case letters among root rot categories within a pathogen water treatment are significantly different at $P \leq 0.05$.

Table 1 Isolation of *Pythium* and *Phytophthora* from rhododendron plants (Rhododendron 'Boule de Neige' trial 1 and 'Scintillation' trial 2 and 3) either not inoculated (Control) or inoculated with *Phytophthora cinnamomi* or *P. plurivora*.

Pathogen Treatment	Trial	Isolation Frequency (% of plants at end of trial)			
		Root Rot ^z			All Plants ^y
		Healthy	Moderate	Severe	
----- <i>Pythium</i> ^x -----					
Control	1	43(12/28)ab	33(4/12) a	-	40(16/40)bc
	2	58(11/19)b	70(14/20)ab	100(1/1)	65(26/40)d
	3	75(9/12) b	82(14/17)b	9(1/11)a	85(34/40)e
<i>P. cinnamomi</i>	1	-	0(0/1)	8(3/39)a	8(3/40) a
	2	-	50(1/2)	5(2/38)a	8(3/40) a
	3	75(3/4) b	62(16/26)ab	60(6/10)b	63(25/40)d
<i>P. plurivora</i>	1	13(2/16) a	42(8/19) a	20(1/5) ab	28(11/40)b
	2	18(3/17) a	35(8/23) a	-	28(11/40)b
	3	50(4/8) ab	63(15/24)ab	63(5/8) b	60(24/40)cd
----- <i>Phytophthora cinnamomi</i> ^w -----					
Control	3	0(0/12) *	0(0/17) a*	18(2/11)A	5(5/40) a*
<i>P. cinnamomi</i>	1	ND	ND	ND	ND
	2	ND	ND	ND	ND
	3	0(0/4)	15(4/26) a*	60(6/10)A	25(10/40)a*
<i>P. plurivora</i>	1	ND	ND	ND	ND
	2	ND	ND	ND	ND
	3	0(0/8) *	0(0/24) a*	50(4/8) A	10(4/40) a*

^zValues are the percentage of plans in each root rot category across all water and damage treatment where either *Pythium* species or *P. cinnamomi* were isolated from the roots. Values in parenthesis are the number of plants with a positive pathogen isolation / total number of plants in each root rot category. Root rot was evaluated 18 WAI in trials 1 and 2 and at 24 WAI in trial 3. Healthy: (>50% of root system with white healthy roots), moderate root rot (<50% of root system with white healthy roots), and severe root rot (>75% of root system with dark, dead roots). ND=not detected.

^yValues are percentage of all plants across water treatments and damage treatments with a positive pathogen isolation (n=40).

^xValues with different lower case letters within a column are significantly different at $P \leq 0.05$.

^wValues in a column denoted with an asterisk (*) are significantly different than those for *Pythium* in trial 3. *P. cinnamomi* was isolated from noninoculated and *P. plurivora* plants only in trial 3.

Table 2 Relationship between root rot and oomycete isolation in rhododendron plants (Rhododendron 'Boule de Neige' trial 1 and 'Scintillation' trial 2 and 3) either not inoculated (Control) or inoculated with *Phytophthora cinnamomi* or *P. plurivora*.

Trial and Pathogen Treatment	Isolation Frequency (% of plants at end of trial)				
	Root Rot ^z			All Plants ^y	
	Healthy	Moderate	Severe		
Trial 1					
Control	0 (0/28)	0 (0/12)	-	0 (0/40)	A
<i>P. cinnamomi</i>	-	100 (1/1) b	97 (38/39) a	98 (39/40)	C
<i>P. plurivora</i>	25 (4/16) a	73 (14/19) b	100 (5/5) b	58 (23/40)	B
Trial 2					
Control	0 (0/19)	0 (0/20)	0 (0/1)	0 (0/40)	A
<i>P. cinnamomi</i>	-	100 (2/2)	100 (38/38)	100 (40/40)	C
<i>P. plurivora</i>	12 (2/17) a	22 (5/23) a	-	18 (7/40)	B
Trial 3					
Control	0 (0/12)	0 (0/17) a	18 (2/11) a	5 (2/40)	A
<i>P. cinnamomi</i>	0 (0/4) ab	15 (4/26) b	60 (6/10) a	25 (10/40)	B
<i>P. plurivora</i>	25 (2/8) a	25 (6/24) a	62 (5/8) a	33 (13/40)	B

^zValues are percentage of plants in each root rot category across all water and damage treatments with a positive pathogen isolation and values in parenthesis is the number of plants with a positive pathogen isolation / total number of plants in each root rot category. Root rot was evaluated at 18 weeks after inoculation (WAI) for trial 1 and 2 and at 24 WAI for trial 3. Healthy (>50% of root system with white healthy roots), moderate root rot (<50% of root system with white healthy roots), and severe root rot (>75% of root system with dark, dead roots).

^yValues are percentage of all plants across water treatments and damage treatments with a positive pathogen isolation (n=40). Values with different lower case letters within a row or upper case letters within a column are significantly different at P <0.05.

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Chapter 3 Biomass of rhododendrons is greater with added N but so is disease severity when plants are infected with *P. cinnamomi* but not *P. plurivora*.

Abstract

Phytophthora root rot decreases availability and quality of rhododendrons produced in the USA. Symptoms of Phytophthora root rot include root necrosis, leaf chlorosis, stunting, and permanent wilt. *Phytophthora plurivora* and *P. cinnamomi* have been isolated from symptomatic stems and roots and frequently occur in surveys of Oregon nurseries and may play an important role in disease. Many studies show that high plant nitrogen (N) status increases Phytophthora disease severity in other hosts, but there is no information on the effect of elevated N on Phytophthora root rot caused by *P. cinnamomi* or *P. plurivora* in rhododendrons. In order to better understand the impact of N and pathogen species on root rot development, *Rhododendron catawbiense* 'Boursault' was grown with no (0 g N/pot), low (1.04 g N/pot), or high (3.12 g N/pot) rates of N incorporated into the container substrate and infested with either *P. cinnamomi* or *P. plurivora*. Both the low and high N rates changed plant physiology and promoted an increase in biomass and leaf greenness when plants were not inoculated with *Phytophthora*. Nitrogen application enhanced N, K, Mg, P, S, and Mn uptake in low and high N treatments. Differences in growth between plants in the no N treatment and those fertilized with N were primarily driven by N availability and its influence on the uptake of other nutrients and water. Application of N also increased the severity of Phytophthora root rot in plants inoculated with *P. cinnamomi* but not in plants inoculated with *P. plurivora*. While mortality of plants inoculated with *P. plurivora* increased from no N to high N, the difference was not significant. Compared to the no N application,

application at the highest rate increased root rot and mortality (from 10 to 50% and 0 to 10% in *P. plurivora* plants in trials 1 and 2 respectively, and from 20 to 70% and 30 to 90% in *P. cinnamomi* plants in trials 1 and 2 respectively). On average, inoculation with *P. plurivora* did not restrict uptake of any nutrient, but inoculation with *P. cinnamomi* restricted uptake of Mg, Mn, and Cu. Although growers could reduce the amount of root damage by reducing the amount of N applied, plant quality would still be suboptimal as shoot mortality occurred even in the no N treatment and noninoculated control plants in the no N treatment were chlorotic and undersized. As nurseries may apply high amounts of N during production to promote fast growth and economic production, it is important to understand how N affects growth and disease. In order to understand how N (both rate and form) affects disease, more research is needed on different rhododendron cultivars sensitivity to N and effects of N in vitro on *P. cinnamomi* and *P. plurivora*. The effect of increased N via foliar application should also be examined, as elevated N may not increase disease severity if applied away from the site of infection.

Introduction

The nursery and greenhouse industry is the highest valued agricultural commodity in Oregon (Oregon Department of Agriculture 2017). Rhododendrons, a popular ornamental shrub primarily grown for their flowers, are important to the industry (Sleumer 1980b). Oregon rhododendron sales account for a quarter of national sales, a value of \$11 million (USDA National Agricultural Statistics Service 2015). Phytophthora root rot, caused by multiple *Phytophthora* species, is a common nursery disease of rhododendrons that causes significant production losses (Englander et al. 1980; Hoitink

et al. 2014). *Phytophthora plurivora* and *P. cinnamomi* have been isolated from symptomatic stems and roots (Beaulieu et al. 2017; Duan et al. 2008; Kroon et al. 2012) and frequently occur in surveys of Oregon nurseries (Knaus et al. 2015; Parke et al. 2014).

Plant nitrogen (N) status plays an important role in disease. Many studies show that high plant N status increases disease severity. Some studies suggest that high N concentrations in the host changes tissue composition and anatomy to make it more easily penetrable by pathogens (Hoitink et al. 2003; Hummel et al. 2013; Scarlett et al. 2013; Tan et al. 2002; Utkhede and Smith 1995). High tissue N concentrations lower the amount of cellulose in cells, decreasing cell wall thickness (Huber and Thompson 2007). Application of N fertilizer increases plant N concentrations and may increase plant susceptibility to *Phytophthora* diseases. In rhododendrons inoculated with *P. ramorum*, foliar lesion size increased with foliar N concentration (Hummel et al. 2013). Number of foliar lesions and foliar lesion size was positively correlated with N concentration in young foliage of rhododendron inoculated with *P. cactorum* (Hoitink et al. 1986). Mortality of durian (*Durio zibethinus* Murr.) inoculated with *P. palmivora*, which causes root rot in durian, increased with N application rate (Tan et al. 2002). Plant health of *Angophora costata* and *Eucalyptus piperita* infected with *P. cinnamomi*, was lower when fertilized with N (Scarlett et al. 2013). In Scarlett's study, high N was thought to promote root growth that was more susceptible to pathogen penetration. Severity of crown and root rot of apple trees (*Malus domestica*) inoculated with *P. cactorum* increased when fertilized with N (Utkhede and Smith 1995). In Utkhede's study, the N

fertilizer was thought to increase disease severity because plant tissues became more succulent with N application, which favors pathogen penetration.

There are also reports that high plant N status has no effect or decreases disease (Laywisadkul et al. 2010; Lee and Zentmyer 1982; Scheuerell et al. 2005; Zentmyer and Bingham 1956). Some studies suggest that N in the root environment decreases inoculum while other studies suggest that N enhances host defense. Nitrogen from foliar applications of urea did not influence incidence of stem lesions on pear trees (*Pyrus communis* L) inoculated with *P. syringae* (Laywisadkul et al. 2010).

Root rot was not more severe with greater rates of N fertilizer in *Persea indica* and avocado inoculated with *P. cinnamomi* (Lee and Zentmyer 1982; Zentmyer and Bingham 1956). In both studies, high rates of N fertilizer were thought to result in less disease because high N decreased the inoculum level in the root environment. There is no information on the effect of elevated N on disease caused by *P. plurivora*. However, in beech seedlings (*Fagus sylvatica*) inoculated with *P. citricola*, a *Phytophthora* species closely related to *P. plurivora* that causes root rot, mortality was negatively correlated with N application rate (Fleischmann et al. 2010). In Fleischmann's study, while pathogen sporulation was slightly higher with high N in vitro, they hypothesized that the high N rate promoted defensive compounds in the plant. Nurseries apply high amounts of N during production to promote fast growth and economic production (Hoitink et al. 2003). Because the effect of plant N status on disease varies by host and pathogen, individual complexes must be assessed (Huber and Watson 1974).

P. cinnamomi and *P. plurivora* are two of the most common pathogens isolated from diseased plants in Oregon nurseries (Parke et al. 2014). These two pathogens both cause root rot on rhododendron nursery plants (Weiland et al. 2018 in press), but little information is known about how plant N status alters the ability of these pathogens to cause disease. The objective of this study was to assess the impact of increasing N fertilizer rate on the progression and severity of Phytophthora root rot of rhododendron. The experiment involved growing rhododendrons with no, low, or high levels of N incorporated into the container substrate and infested with either *P. cinnamomi* or *P. plurivora*. The hypotheses tested were (1) Greater rates of N fertilizer application will cause more rapid disease progression and increase disease severity; and (2) There will be no difference in disease progression or disease severity between *P. cinnamomi* and *P. plurivora* at different rates of N fertilizer.

Materials and Methods

Experimental design

The experimental design was a full factorial, complete randomized block design with three inoculation treatments (*P. cinnamomi*, *P. plurivora* and a noninoculated control) and three N treatments (no, low, and high N) replicated in 10 blocks. The experiment was repeated for two trials. Both trials ran for 18 weeks after inoculation (WAI). Trials were conducted during 2018 in a greenhouse. Trial 1 inoculation was 18 January 2018 and trial 2 inoculation was 25 January 2018.

Isolate selection

Isolate R057 (*P. cinnamomi*, GenBank Accession No. MG560189) and isolate R003 (*P. plurivora*, MG560192) were collected from diseased plants in Willamette Valley Oregon nurseries that grow rhododendrons in containers. Isolates were identified based on morphology and on internal transcribed spacer (ITS) ribosomal DNA sequences matched at 99% to 100% identity to DNA sequences from type or extype isolates of *P. cinnamomi* (KC478663 and FJ801806) and *P. plurivora* (FJ66522) (Jung and Burgess 2009; Scanu et al. 2013).

Inoculum Preparation

Inoculum was prepared following the method outlined in Chapter 2.

Pre-inoculation fertilizer treatments

Prior to inoculation, plants were fertilized once per week for 4 weeks for trial 1 and for 5 weeks for trial 2 with a modified Hoagland solution (Hoagland and Arnon 1950) containing 0.5 mM potassium phosphate pH 6.0 (KH_2PO_4), 0.5 mM potassium sulfate (K_2SO_4), 1 mM magnesium sulfate ($\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$), 1 mM calcium chloride ($\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$), 0.03 mM 10% Iron DTPA, 4.6 μM manganese (II) chloride tetrahydrate ($\text{MnCl}_2 \cdot 4\text{H}_2\text{O}$), 0.02 mM boric acid (H_3BO_3), 0.4 μM copper (II) sulfate pentahydrate ($\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$), 0.7 μM sodium molybdate dehydrate ($\text{Na}_2\text{MoO}_4 \cdot 2\text{H}_2\text{O}$), and 1.5 μM zinc sulfate heptahydrate ($\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$). No ammonium sulfate ($(\text{NH}_4)_2\text{SO}_4$) was used in fertilizer for the no N treatment. Fertilizers for the low N and high N treatments contained, respectively, 0.01 mM N, and 1 mM N from $(\text{NH}_4)_2\text{SO}_4$.

Inoculation Method

The vermiculite inoculum was added to a soilless substrate (Metro-Mix 840PC, Sun Grow Horticulture, Agawam MA) at a 10% v/v inoculum/substrate at an inoculum level of 100 propagules per gram (ppg). The vermiculite inoculum and substrate were mixed in a cement mixer for 10 min with a NaOCl wash between each pathogen. Noninfested vermiculite was used for the control. One liter of each pathogen and control mix were then mixed by hand with a slow release urea N fertilizer (X-Cote 43-0-0 polymer-coated sulfur-coated urea, J.R. Simplot Company, Lathrop CA) at one of three rates: no (0 g N/ pot), low (1.04 g N/pot), and high (3.12 g N/pot). The rhododendron plant was placed directly above the 1 L of inoculum or noninoculated control substrate/vermiculite/fertilizer mix in 2.5 liter polyethylene containers (black Poly-Tainer PT NS300, BFG Supply, Burton OH). Infested or noninfested soilless substrate without N fertilizer was packed around the plant to 2.5 cm below the container rim.

Rhododendron catawbiense 'Boursault' (RHS #58) was used for both trials. This cultivar is susceptible to *P. cinnamomi* (Hoitink and Schmitthenner 1970; Hoitink and Schmitthenner 1975), but its susceptibility to *P. plurivora* is unknown. Plants were obtained as rooted cuttings in L2 liner trays in October 2017. Plants were kept outdoors in a canyard, then brought into the greenhouse to acclimate for approximately 2 months prior to inoculation. Ten representative plants were sampled for initial biomass and for contamination of the roots by *Phytophthora* or *Pythium* species prior to inoculation by removing five one centimeter root segments per root ball, sterilizing in 70% ethanol for 30 s, rinsing in distilled water and plating on PARP. Plants were \approx 1 year old at the

beginning of the experiment with an average total plant dry weight of 2.56 g. No pathogens were isolated from roots prior to inoculation.

Greenhouse conditions and supplemental fertilizer applications

Greenhouse temperature was set to approximately 15°C night and 18°C day. Sixteen hours of supplemental light were provided daily (LumiGrow ES330, Lumigrow, Emeryville CA), starting 5 days after inoculation. Blue and red LEDs were set at full intensity. There were approximately 0.59 lights/m² above greenhouse benches (7 lights over a 1.8 m x 6.4 m bench). Environmental sensors read air temperature, relative humidity (VP 4 Humidity/Temp/Barometer, Decagon Devices, Pullman WA) and light (QSO-S PAR Photon Flux Sensor, Decagon Devices, Pullman WA) and the data were logged hourly (EM50, Decagon Devices, Pullman WA) (Fig A 4).

Daily averages PAR, T_{air} , and RH for trials 1 and 2 were 207 $\mu\text{mol s}^{-1} \text{m}^{-2}$ (1437 $\mu\text{mol s}^{-1} \text{m}^{-2}$), and 210 $\mu\text{mol s}^{-1} \text{m}^{-2}$ (1437 $\mu\text{mol s}^{-1} \text{m}^{-2}$), 22°C (10°C min and 30°C max) and 23°C (10°C min and 30°C max), and 52% (15% min and 81% max for both trials).

Leaf color was monitored weekly by taking measurements of three fully expanded leaves per plant from the current season's growth (Dualex Scientific sensor, Orsay Cedex France). All plants were fertilized several times during the trials, in lieu of water, with a N-free fertilizer to address interveinal chlorosis (15 ml/3.78 L Liquinox Bloom 0-10-10, Liquinox CO., Orange CA). Fertilizer was applied once a week with 0.14 g P and 0.08 g K at 5, 9, 11 and 13 WAI, and 0.28 g P and 0.15 g K at 15 and 17 WAI.

All plants were watered twice daily at 8 am and 2 pm using emitters with a 1 gallon per hour flow rate (1 emitter per pot; 1 GPH Pressure Compensating PC Drip

Emitter, DIG Corporation, Vista CA). At each irrigation event, plants were watered for 1 min (1 to 14 WAI) or 2 min (15 to 18 WAI). Irrigation was managed to achieve a volumetric water content of $0.3 \text{ m}^3/\text{m}^3$, corresponding to approximately 70% container capacity. Electrical conductivity (EC) and volumetric water content in the growing substrate were monitored using buried probes connected to data loggers (5TE Sensor, Decagon Devices, Pullman WA) on 3 replicates in each treatment.

Disease and plant health evaluation

Disease and plant health were evaluated according to methods outlined in chapter 2 with the addition of plant nutrient analysis and estimation of disease progression using the area under the disease progress curve (AUDPC). After plants were harvested and dry weight (dw) was measured, the dried plant parts were ground to pass through a 40-mesh (425- μm) screen and analyzed for C and N using a combustion analyzer (TruSpec CN; Leco Corp., St. Joseph MI), P, K, Ca, Mg, S, Fe, B, Cu, Mn, Zn, and Na using ICP-OES (Optima 3000DV; Perkin Elmer, Wellesley MA) following microwave digestion in 70% (v/v) nitric acid (Gavlak et al. 2005; Jones et al. 1990). Reference standard apple (*Malus domestica*) leaves (no. 151, National Institute of Standards and Technology, Gaithersburg MD) were included in each set of samples to ensure accuracy of instrument and digestion procedures. Elemental content of each plant part was calculated by multiplying the dw of a given part by the concentration of each element therein (Chapin and Van Cleve 1989). Total plant content was calculated by adding the content of a specific element from each plant part. Total plant concentrations of each element was calculated by dividing plant content by plant dw. To

adjust for treatment effects of plant size, uptake of each nutrient in pathogen inoculated plants was calculated relative to non-inoculated controls and uptake of each nutrient in low N and high N was calculated relative to plants in the no N treatment.

Statistical analysis

All data were analyzed using Statistica, version 13 (TIBCO Software Inc., Palo Alto CA). Frequency data (visual symptoms, root rot, and pathogen isolation) were analyzed using generalized linear models. For each week, a binomial distribution with logit link function was used for analyzing wilt, mortality, and pathogen isolation data and an ordinal multinomial distribution with logit link function was used for the analysis of root rot data. Models for generalized linear analyses included block with trial, pathogen treatment, and N treatment as main effects in a full factorial design. Week was included as a within subject parameter for analysis of mortality. Forward and backward stepwise selection was used for model selection and effects evaluated using type 1 LR test and differences among treatments evaluated using predicted means with 95% confidence intervals and Yates corrected Chi square (Mantel 1974) and Fisher's exact test (Agresti 1992) at $P < 0.05$. Data are presented as the frequency of plants in each category.

For all other data, normality was assessed by examining normal P-P plots (Neter et al. 1985) and equal variance was assessed by Levene's test for homogeneity of variance (Glass and Hopkins 1996). Biomass, leaf color, stomatal conductance, nutrient concentration, and AUDPC were evaluated with Kruskal Wallis ANOVA and Median Test, and differences among means were assessed at $P < 0.05$ (Siegel and Castellan 1988). Volumetric water content was analyzed using a repeated measures ANOVA with

block in the model and trial, pathogen, N treatment, and WAI as main effects in a full factorial design. Means of significant effects from ANOVA were evaluated using the Greenhouse-Geisser correction and the Huynh-Feldt correction at $P < 0.05$ (Greenhouse and Geisser 1958, 1959; Huynh and Feldt 1970).

Results

Noninoculated controls

Greater rate of N incorporation generally resulted in higher biomass in noninoculated (control) plants (Fig. 8), higher leaf greenness, and lower leaf redness (Fig. 9). In control plants, chlorophyll values fluctuated slightly in low N and high N treatments during the trials but were fairly consistent in the no N treatment (Fig. 10). Anthocyanin values were fairly consistent among N treatments in control plants during the trials (Fig. 11). Control plants fertilized with N generally had higher stomatal conductance (g_s) than plants in the no N treatment (Fig. 12).

There was no wilt or death in control plants (Fig. 13). No control plants had symptoms of root rot (Fig. 14) and neither *P. cinnamomi* nor *P. plurivora* were isolated from control plants. However, *Pythium* was isolated from 2% of roots (1/60) of control plants.

Compared to plants at the beginning of the experiment, when plants were not fertilized with N, there was significant uptake of all nutrients by 18 WAI in noninoculated controls (Table 3). Nitrogen application enhanced N, K, Mg, P, S and Mn uptake by control plants in the low N and high N treatment (greater biomass, concentration, and content) (Fig. 15). For many other nutrients, N application increased nutrient uptake by

control plants, but differences in total plant content were a result of greater plant biomass and did not cause a significant increase in concentration (e.g. C, Ca, B, Cu, Fe, and Zn in low and high N plants). In general, the effects of N application on nutrient uptake by control plants was reflected in concentrations of N, P and Mn in all plant structures, K and Mg in leaves and stems, and S in leaves and roots

P. plurivora

Total plant biomass and g_s of plants inoculated with *P. plurivora* were similar to control plants in each N treatment (Fig. 8 and Fig. 12). Leaf greenness and leaf redness in *P. plurivora* inoculated plants were similar to controls (Fig. 9) and fluctuated more than controls after application of N free P-K fertilizer (Fig. 10 and Fig. 11).

Beginning 5 or 6 WAI, a greater number of *P. plurivora* inoculated plants began to wilt and die compared to controls (Fig. 13). Compared to control plants in the high N treatments, plants inoculated with *P. plurivora* in the same N treatments had greater wilt and mortality in trial 1.

P. plurivora inoculated plants also had less healthy roots than controls, but this was not affected by N application. By 18 WAI, 17% (10/60) of roots had severe root rot across all three treatments (Fig. 14). *P. plurivora* was isolated from 20% (10/60) of roots and 23% of stems (14/60). Root contamination of *P. plurivora* inoculated plants by *Pythium* was low and similar to the amount observed in controls at 3% (2/60) ($P=1.000$).

Plants inoculated with *P. plurivora* had similar nutrient uptake as controls, even though prior to symptom expression (4 WAI), EC values of *P. plurivora* inoculated plant substrate were greater (≈ 2 mS/cm) than that of control substrate in low and high N

treatments (Fig A 6). Compared to plants at the beginning of the experiment, when plants were not fertilized with N, there was significant uptake of all nutrients by 18 WAI in plants inoculated with *P. plurivora* (Table 3). *P. plurivora* inoculated plants had similar nutrient uptake as controls when plants were not fertilized with N. However, *P. plurivora* inoculated plants had lower Zn content than controls when plants were not fertilized, but not Zn concentration (concentration data not shown). On average, inoculation with *P. plurivora* did not restrict uptake of any nutrient (Fig. 15). Although total plant concentration of several nutrients was lower in *P. plurivora* inoculated plants than controls plants, nutrient content was similar. Similarly, when *P. plurivora* inoculated plants had a greater concentration of specific nutrients than controls, nutrient content was similar. Similar to controls, N incorporation to *P. plurivora* inoculated plants enhanced N, K, Mg, P, S and Mn uptake in the low N and high N treatment (Fig. 15).

P. cinnamomi

In both trials, disease symptoms caused by *P. cinnamomi* were more severe than with *P. plurivora*, and higher in severity with higher N rates. In contrast to controls and *P. plurivora* inoculated plants, N application did not increase total plant biomass of *P. cinnamomi* inoculated plants and *P. cinnamomi* inoculated plants had the lowest biomass in any N treatment (Fig. 8). Within each N treatment, leaf greenness in *P. cinnamomi* inoculated plants was similar to that of controls and *P. plurivora* inoculated plants and fluctuated more than controls and *P. plurivora* inoculated plants after application of N free P-K fertilizer (Fig. 9 and Fig. 10). Leaf redness of *P. cinnamomi* inoculated plants was similar to that of controls and *P. plurivora* inoculated plants in all

N treatments (Fig. 9). Leaf redness fluctuated more than controls and *P. plurivora* inoculated plants after application of N free P-K fertilizer (Fig. 11). In low N and high N, g_s was lower in *P. cinnamomi* inoculated plants than in controls and *P. plurivora* inoculated plants (Fig. 12).

Beginning 4 WAI, a greater number of *P. cinnamomi* inoculated plants began to wilt and die compared to controls (Fig. 13). Compared to control plants and *P. plurivora* inoculated plants, *P. cinnamomi* inoculated plants had greater wilt and equal or greater mortality in the high N treatment (Fig. 13). Disease progressed more rapidly in *P. cinnamomi* inoculated plants than *P. plurivora* inoculated plants and more rapidly in high N than no N (Table 4).

P. cinnamomi inoculated plants had less healthy roots than controls and *P. plurivora* inoculated plants, but this was not affected by N application. By 18 WAI, 95% (57/60) of roots across all treatments had severe rot and 5% (3/60) had moderate rot (Fig. 14). The pathogen was isolated from 93% (56/60) of roots and 90% (54/60) of stems. There was greater isolation of *P. cinnamomi* from *P. cinnamomi* inoculated plants than *P. plurivora* from *P. plurivora* inoculated plants in both roots and stems ($P<0.000$). *Pythium* was isolated more frequently from the roots of *P. cinnamomi* inoculated plants (15% or 9/60 plants) than control plants ($P=0.021$). Although *Pythium* was isolated more frequently from *P. cinnamomi* inoculated plants than *P. plurivora* inoculated plants, the difference was not significant ($P=0.058$).

Compared to controls and *P. plurivora* inoculated plants, inoculating plants with *P. cinnamomi* reduced uptake and translocation of specific nutrients. Prior to symptom

expression (week 4), EC values of *P. cinnamomi* plant substrate was greater (≈ 2 mS/cm) than that of controls and similar to *P. plurivora* inoculate plant substrate EC values in treatments low and high N (Fig A 6). Compared to plants at the beginning of the experiment, when plants were not fertilized with N, there was no significant N, P, or Mg uptake by 18 WAI in plants inoculated with *P. cinnamomi* (Table 3). When plants were not fertilized with N, plants inoculated with *P. cinnamomi* had lower nutrient uptake than controls for all nutrients and similar nutrient uptake as *P. plurivora* inoculated plants for all nutrients. On average, inoculation with *P. cinnamomi* restricted uptake of three nutrients, Mg, Mn, and Cu (Fig. 15). The effects of *P. cinnamomi* on uptake decreased accumulation of Mn and Mg in all plant parts, Ca, S, and B in leaves, K in leaves and roots, and Cu, P, and Zn in roots. Although, total plant concentration of several nutrients was greater in *P. cinnamomi* inoculated plants than controls and *P. plurivora* inoculated plants, total plant content was similar or lower than controls and *P. plurivora* inoculated plants. Similar to controls and *P. plurivora* inoculated plants, N application to *P. cinnamomi* inoculated plants enhanced N, K, Mg, P, S and Mn uptake in the low N and high N treatment.

Discussion

Rate of N incorporation into growing media alters plant growth and function of rhododendron. Nitrogen promotes plant growth (Huber and Thompson 2007; Marty et al. 2009). Application of N at both the low and high rate changed plant physiology and resulted in an increase in biomass and leaf greenness when plants were not inoculated with *Phytophthora*. Leaf anthocyanin values were lower with added N, indicating that

plants were less stressed. In contrast, noninoculated control plants grown with no N were small and chlorotic. Plants grown with no N also had high anthocyanin values and low stomatal conductance compared to plants grown with either rate. High anthocyanin values may be indicative of pathogen infection or environmental stress (i.e. water, nutrient, substrate EC, and temperature stress) (Gitelson and Merzlyak 2004). Substrate VWC was consistent among N treatments and maintained at $\approx 70\%$ container capacity. It is possible that plants in the no N treatment experienced water stress as well as nutrient stress. Rhododendrons grown without N have a lowered ability to absorb water and nutrients and may respond to water stress by stomatal closure (Scagel et al. 2011a). While rhododendrons differ in their sensitivity to N (Shelton 1967), noninoculated controls in the current study did not exhibit symptoms of excess N application (i.e. leaf burn) even at the highest N application rate. Fertilizer application is one of the primary drivers of substrate EC and pH. EC values of controls in high N remained within the recommended EC range for containerized rhododendrons, which is below 1 ds/m (1 mS/cm) (Rouse 1984). This is important because high EC injures roots by decreasing water potential outside the plant, which leads to water stress and overwhelms the capacity of the vacuoles to store salts, which leads to decreased movement of carbohydrates and hormones needed for growth (Kozlowski 1997).

All control roots were healthy 18 WAI and were able to absorb nutrients. Even noninoculated controls grown with no N had significant uptake of all nutrients 18 WAI compared to plants at the beginning of the experiment. Interactions between N and other nutrients is common (Huber and Thompson 2007). Nitrogen application enhanced

N, K, Mg, P, S, and Mn uptake in low and high N treatments. These nutrients may be needed in greater amounts to assimilate and translocate added N (Scagel et al. 2008). The results from the current study suggest that differences in growth between plants in the no N treatment and those fertilized with N were primarily driven by N availability and its influence on the uptake of other nutrients and water.

Increased nitrogen rate resulted in greater severity of *Phytophthora* root rot in *P. cinnamomi* inoculated plants but not in *P. plurivora* inoculated plants. Disease severity and rate of disease progression in plants inoculated with *P. cinnamomi*, but not *P. plurivora*, was greater with added N. In contrast to controls, greater rate of N application did not decrease leaf anthocyanin values in *Phytophthora* inoculated plants. Anthocyanin production may be induced by stress from pathogen infection or high substrate EC (Gitelson and Merzlyak 2004). Nitrogen application also resulted in mortality from 10 to 50% and 0 to 10% in *P. plurivora* inoculated plants in trials 1 and 2, respectively, and from 20 to 70% and 30 to 90% in *P. cinnamomi* plants in trials 1 and 2, respectively. Root rot was expected in plants inoculated with these pathogens because previous studies showed that both can cause severe root rot in rhododendrons (Lilja et al. 2011; Schoebel et al. 2014; Weiland et al. 2018 in press). Previous studies with *P. cactorum* and *P. ramorum* have shown that increasing N fertilizer rate is associated with greater disease in rhododendrons inoculated with *Phytophthora* (Hoitink et al. 1986; Hummel et al. 2013). However, our study is the first time that shows root rot is influenced by N fertilizer in plants inoculated *P. cinnamomi*.

In Weiland's 2018 study, mortality of 'both Yaku Princess' and 'Cunningham's White' inoculated with *P. cinnamomi* was greater from trial 1 to 3 while the rate of incorporated fertilizer prior to inoculation also increased from trial 1 to 3. While Weiland's study did not test for differences in fertilizer rates on disease, there was an observed association between increased fertilizer and greater mortality for both cultivars. In contrast mortality of 'Yaku Princess' inoculated with *P. plurivora* increased from trial 1 to 3 while mortality of 'Cunningham's White' was similar across trials indicating that the effect of increased nutrition on disease may be cultivar as well as pathogen specific. These data support the assertion that individual complexes must be assessed, as the relationship between diseases and N fertilizer rates can differ between cultivars and pathogens (Huber and Watson 1974). To test this, 'Yaku Princess' and 'Cunningham's White' would need to be treated with increasing rates of fertilizer and inoculated with these pathogens to assess differences in disease severity. In addition, plants could also be pretreated with different rates of fertilizer prior to inoculation and fertilizer incorporation in order to have differences in host physiology prior to pathogen inoculation.

The effect of N form on pathogen assimilation and on disease varies. There is preliminary evidence that *Phytophthora* species belong to three groups that differ in their ability to assimilate N: group 1 assimilates nitrate efficiently, group 2 assimilates ammonium and organic N efficiently, and group 3 requires organic N (Hohl 1983). Many *Phytophthora* species metabolize inorganic N sources (e.g. NH_4^+ and NO_3^-), but generally have improved growth with organic N sources. The form of N may increase

disease severity more than the rate of N (Huber and Watson 1974; Lee and Zentmyer 1982; Utkhede and Smith 1995). Disease severity in *Persea indica* inoculated with *P. cinnamomi* was lower when N was applied as ammonium sulfate at 300 µg N/g soil (89% root rot at 100 µg N/g soil versus 33% root rot at 300 µg N/g soil) but was similar across calcium nitrate rates (49% root rot at 100 µg N/g soil versus 38% root rot at 300 µg N/g soil) (Lee and Zentmyer 1982). Citrus seedlings inoculated with *P. citrophthora* or *P. parasitica* had increased susceptibility with N applied as ammonium sulfate or urea (47 to 59% of roots infected) than with N applied as nitrate (3% of roots infected) (Huber and Watson 1974). Crown and root rot severity of apple trees (*Malus domestica* Borkh.) inoculated with *P. cactorum*, increased with added N in the form of ammonium nitrate, and was unaffected with added N in the form of monoammonium phosphate (Utkhede and Smith 1995). Root rot severity of avocados (cv Edranol) inoculated with *P. cinnamomi* was greater with N applied in the form of urea (rating of 5) and nitrate (rating of 4.9) versus with N applied in the form of ammonium sulfate (rating 4.1) where root rot rating of 5 = root rot symptoms on more than 80% of root area and 4 = root rot symptoms on 61 to 80% of root area (Duvenhage et al. 1992). Changing the form of N will have little effect if the host is highly resistant or highly susceptible (Huber and Watson 1974).

In the current study, N was applied in the form of ammonium sulfate in the pretreatment and in the form of urea during inoculation. N application in the form of ammonium or urea does not increase growth of *P. cinnamomi* compared to unamended media without nitrogen (Duvenhage et al. 1992). Assuming that the *P. cinnamomi*

isolate in the current study behaves similarly to that used in Duvenhague's study, and that conditions for growth are similar to those causing disease, it is likely that increase in disease in plants inoculated with *P. cinnamomi* was due to effects of N application on rhododendron (i.e. more succulent tissue or change in defense response).

Root rot in *Rhododendron catawbiense* 'Boursalt' caused by *P. plurivora* does not influence nutrient uptake. Prior to symptom expression, EC values of *P. plurivora* substrate was greater than that of controls, suggesting a difference in root physiology and the plants ability to uptake nutrients. However, the nutrient analysis of plant material at the end of the experiment showed that *P. plurivora* did not restrict the uptake of any nutrient and *P. plurivora* also did not decrease plant biomass. Although mortality of *P. plurivora* inoculated plants reached 10 to 50% in the high N treatment, only 17% of roots had severe root rot. This can be explained by how mortality was assessed. If at least one stem died, then the plant was considered dead. Plants differed in the number of stems. Root health of a multi-stemmed plant with one dead stem was different than root health of a single-stemmed plant with one dead stem.

Root rot caused by *P. cinnamomi* restricts nutrient and water uptake and plant growth. Greater rate of N application did not increase biomass or decrease many disease symptoms in plants inoculated with *P. cinnamomi* (i.e. leaf redness and stomatal conductance). The relative lack of response of these measured variables in *P. cinnamomi* plants to N rate can be explained by loss of root function to absorb nutrients and water due to root rot (93% of plants had severe root rot). The high chlorophyll values of *P. cinnamomi* leaves may be explained by differences in nutrient allocation. *P.*

cinnamomi plants had fewer vegetative flushes that would cause changes in leaf color. Leaf anthocyanin values of plants inoculated with *P. cinnamomi* were greater than those of controls and *P. plurivora* plants in treatments low N and high N and fluctuated more abruptly following application of N free P-K fertilizer, indicating plant stress with fertilizer application. Stomatal conductance of *P. cinnamomi* inoculated plants was less than controls or other pathogen treatments and not responsive to N rate, indicating plants were under water stress. Even prior to symptom expression, EC values of *P. cinnamomi* substrate was greater than that of controls, suggesting a difference in root physiology and the plants ability to uptake nutrients. The nutrient analysis showed that plants inoculated with *P. cinnamomi* had a decreased ability to uptake and translocate certain nutrients.

High disease incidence was expected in *Rhododendron catawbiense* 'Boursult' inoculated with *P. cinnamomi*. The seed parent of this cultivar is *R. catawbiense* and belongs to the subgenus *Hymenanthus* (the pollen parent is unknown.) There is less than 3% frequency of resistance to *P. cinnamomi* in this subgenus and the inheritability of resistance is low (Krebs 2013). This type of information on cultivar susceptibility is not available for *P. plurivora*. Results from this study indicate that the cultivar used is more susceptible to *P. cinnamomi* than *P. plurivora* and that susceptibility is greater with increasing N rate only in plants inoculated with *P. cinnamomi* but not *P. plurivora*.

Results partially support hypothesis 1 and fail to support hypothesis 2. Results show that (1) Greater rates of N fertilizer application had more rapid disease progression and higher disease severity but only in plants inoculated with *P. cinnamomi*;

and (2) There were differences in disease progression and disease severity between *P. cinnamomi* and *P. plurivora* at different rates of N fertilizer.

Nitrogen plays an important role in plant growth and in disease. Results from this study indicate that while added N promotes growth in *R. catawbiense* 'Boursalt' not inoculated with *Phytophthora*, disease severity is greater in plants inoculated with *P. cinnamomi* but not *P. plurivora* with added N. Mortality in treatment high N was $\geq 70\%$ in plants inoculated with *P. cinnamomi* and $\geq 10\%$ in plants inoculated with *P. plurivora*. Although reducing N application would reduce disease severity, the quality of plants inoculated with either pathogen would be suboptimal as shoot mortality still occurred in the no N treatment. Decreasing plant N status only slowed the disease. As nurseries may apply high amounts of N during production to promote fast growth and economic production, it is important to understand how N affects growth and disease. In order to understand how N (both rate and form) affects disease, more research is needed on different rhododendron cultivars sensitivity to N and effects of N in vitro on *P. cinnamomi* and *P. plurivora*. The effect of increased N via foliar application should also be examined, as disease severity may not be greater with added N if N is applied away from the site of infection (Huber and Thompson 2007).

Figures and Tables

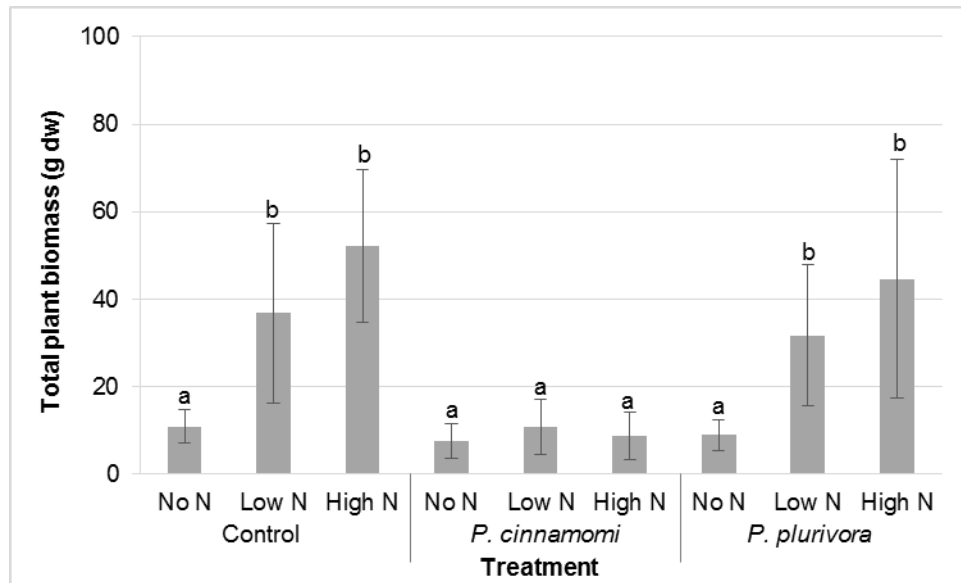


Fig. 8 Total plant biomass of *Rhododendron catawbiense* 'Boursault' plants not inoculated (control) or inoculated with *P. cinnamomi* or *P. plurivora* and grown with three rates of nitrogen (N) for 18 weeks after inoculation. At inoculation, plants received either 0g N/pot slow release N fertilizer (No N), 1.04 g N/pot (Low N) or 3.12 g N/pot (High N). Columns are means across all trials and error bars are mean standard deviations (n=20). Columns with different letters denote significant differences among all pathogen x N rate treatments at $P \leq 0.05$.

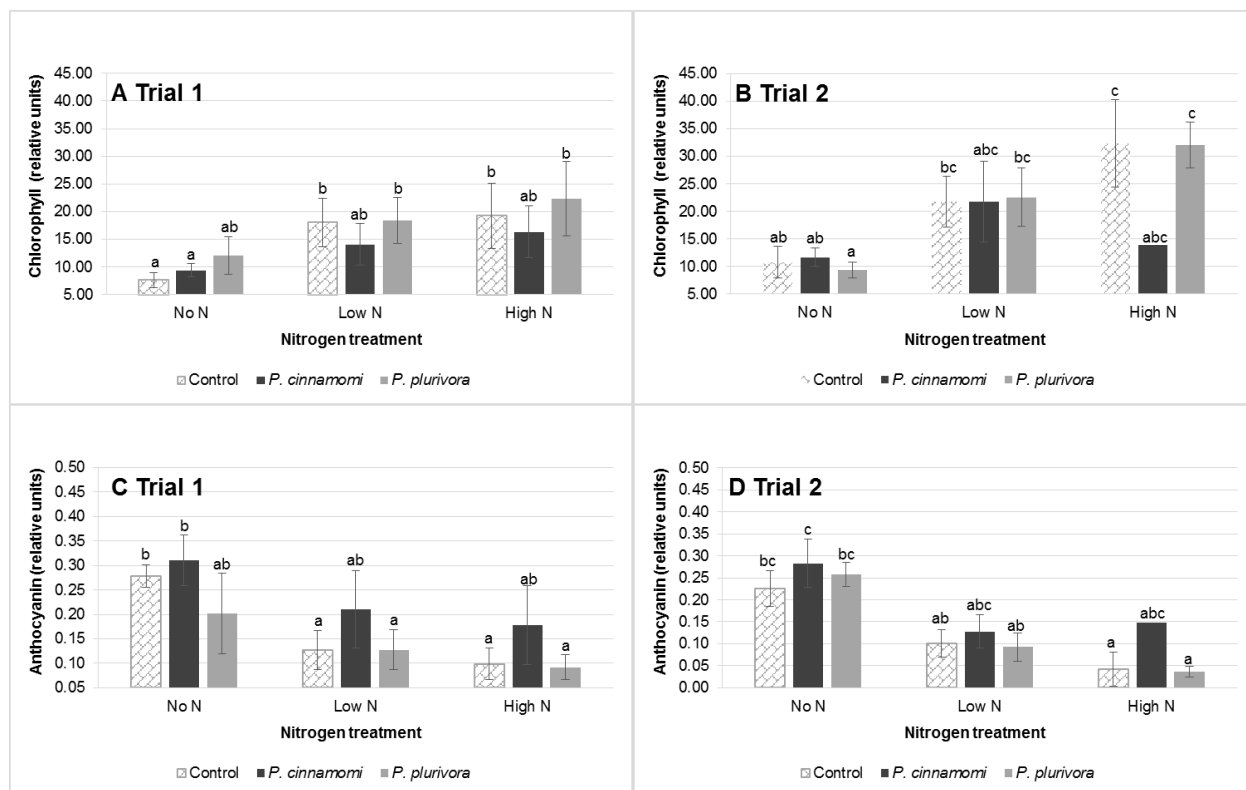


Fig. 9 Chlorophyll (leaf greenness) and anthocyanin (leaf redness) values of *Rhododendron catawbiense* 'Boursault' plants either not inoculated (control) or inoculated with *P. cinnamomi* or *P. plurivora* and grown with three rates of nitrogen (N) for 18 weeks after inoculation (WAI) in trial 1 (A and C) and trial 2 (B and D). At inoculation, plants received either 0 g N/pot slow release N fertilizer (No N), 1.04 g N/pot (Low N) or 3.12 g N/pot (High N). Values are means at 18 WAI and error bars are mean standard deviations (n=10 max). Columns with different lower case letters denote significant differences among treatments at $P \leq 0.05$.

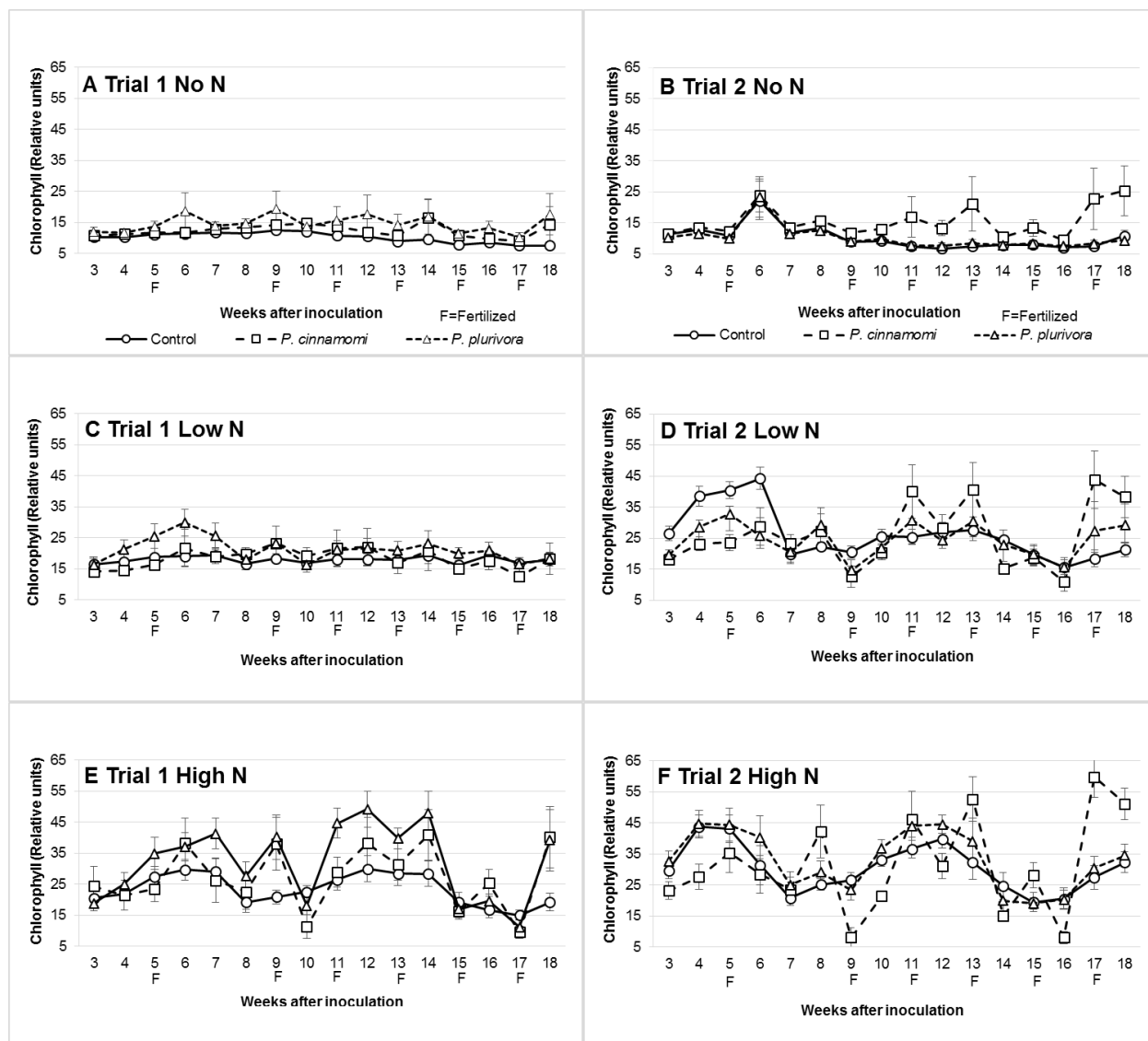


Fig. 10 Chlorophyll (leaf greenness) values of *Rhododendron catawbiense* 'Boursault' plants either not inoculated (control) or inoculated with *P. cinnamomi* or *P. plurivora* and grown with three rates of nitrogen (N) for 18 weeks after inoculation (WAI) in trial 1 (**A**, **C**, **E**) and trial 2 (**B**, **D**, **F**). At inoculation, plants received either 0 g N/pot slow release N fertilizer (No N), 1.04 g N/pot (Low N) or 3.12 g N/pot (High N). Data points are means and error bars show 95% confidence intervals (n=max 10). Weeks when plants were fertilized with N free P-K fertilizer denoted by F below x-axis.

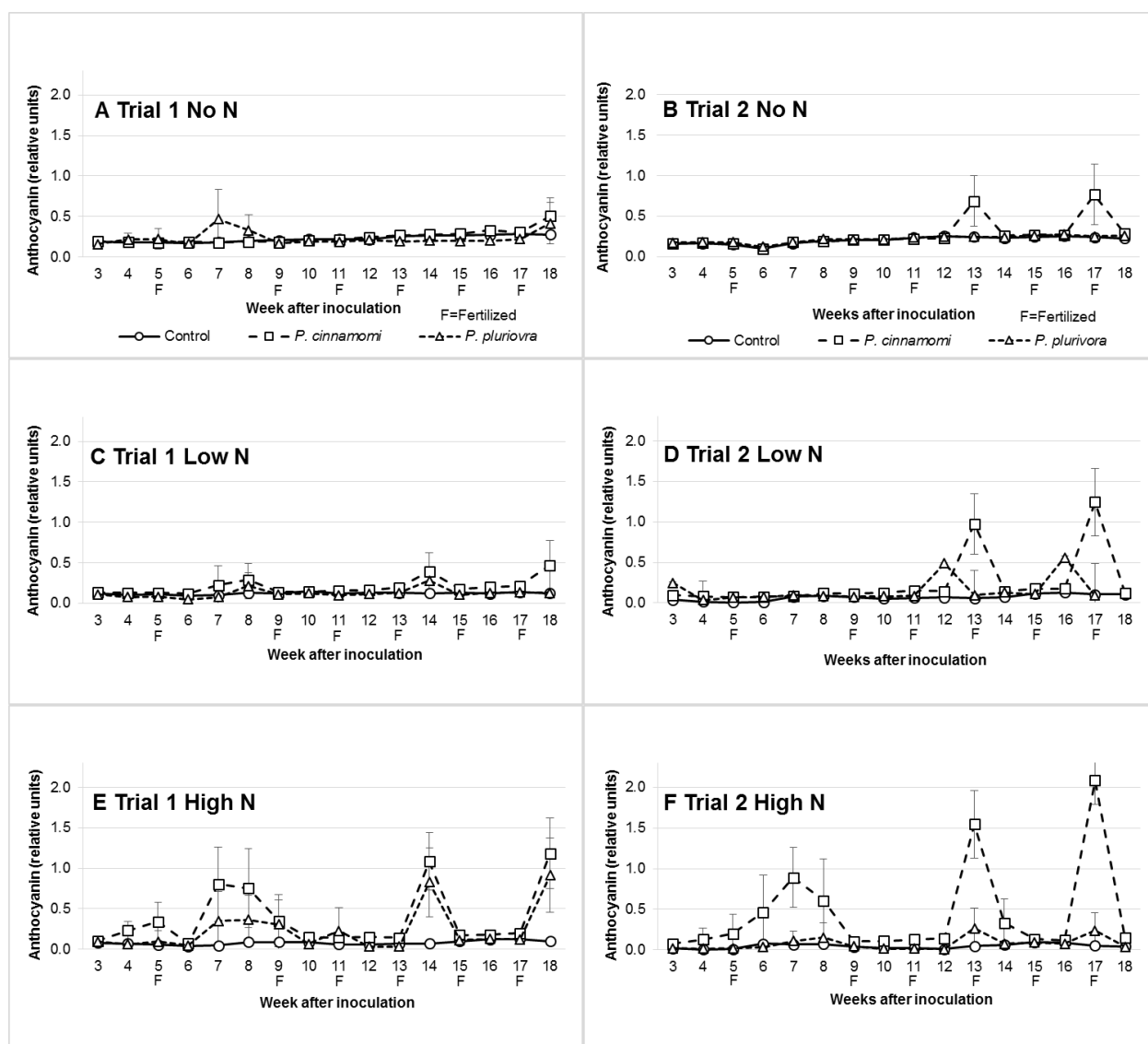


Fig. 11 Anthocyanin (leaf redness) values of *Rhododendron catawbiense* ‘Boursault’ plants either not inoculated (control) or inoculated with *P. cinnamomi* or *P. plurivora* and grown with three rates of nitrogen (N) for 18 weeks after inoculation (WAI) in trial 1 (A, C, E) and trial 2 (B, D, F). At inoculation, plants received either 0 g N/pot slow release N fertilizer (No N), 1.04 g N/pot (Low N) or 3.12 g N/pot (High N). Data points are means and error bars are 95% confidence intervals (n=max 10). Weeks when plants were fertilized with N free P-K fertilizer denoted by F below x-axis.

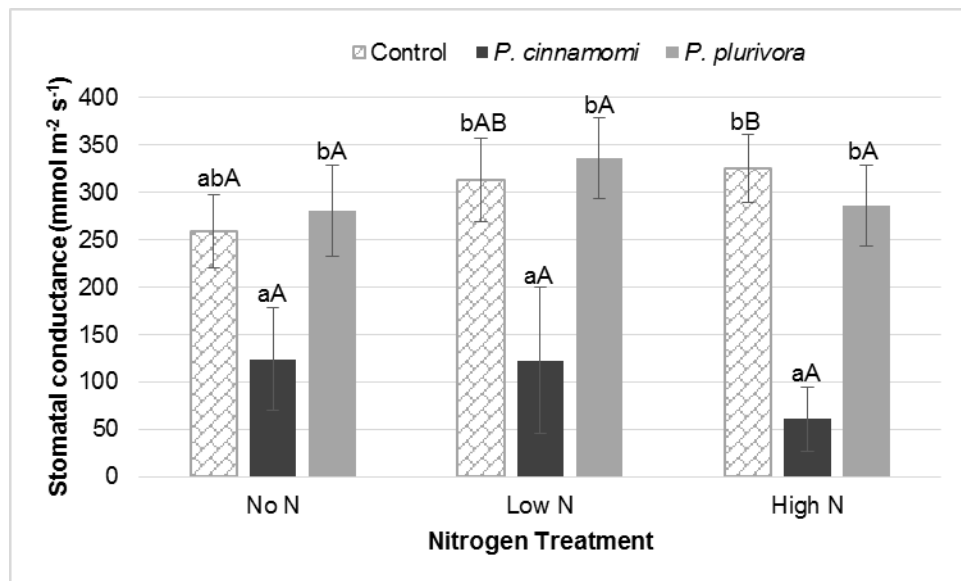


Fig. 12 Stomatal conductance of *Rhododendron catawbiense* 'Boursault' plants either not inoculated (control) or inoculated with *P. cinnamomi* or *P. plurivora* and grown with three rates of nitrogen (N). At inoculation, plants received either 0 g N/pot slow release N fertilizer (No N), 1.04 g N/pot (Low N) or 3.12 g N/pot (High N). Measurements were taken 18 weeks after inoculation (WAI). Values are means across trials (n=max 20). Error bars are standard deviations. Columns with different lower case letters among pathogens within an N treatment and upper case letters among N treatments within a pathogen are significantly different at $P \leq 0.05$.

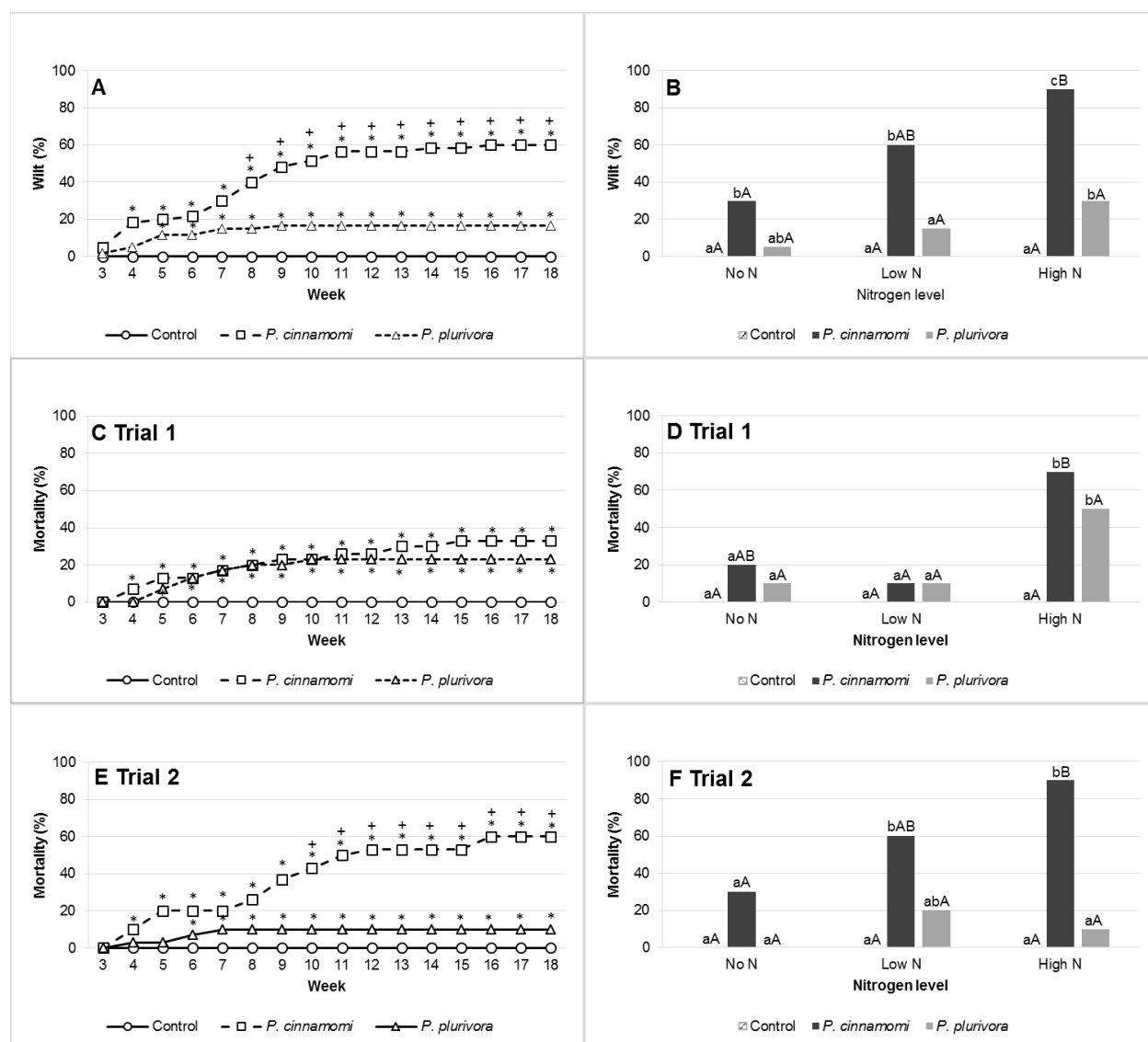


Fig. 13 Wilt and mortality in *Rhododendron catawbiense* 'Boursault' plants either not inoculated (control) or inoculated with *P. cinnamomi* or *P. plurivora* and grown with three rates of nitrogen (N). At inoculation, plants received either no slow release N fertilizer (No N), 1.04 g N/pot (Low N) or 3.12 g N/pot (High N). **(A)** Values are means across all N treatments and trials (n=60). * indicate at which week pathogen became significantly different from the control at $P \leq 0.05$. + indicate at which week pathogens became significantly different from each other at $P \leq 0.05$. **(B)** Values are means at 18 WAI across both trials (n=20). **(C and E)** Values are means across all N treatments (n=30). * indicate at which week pathogen became significantly different from the control at $P \leq 0.05$. + indicate at which week pathogens became significantly different from each other at $P \leq 0.05$. **(D and F)** Values are means at 18 WAI in each trial (n=10). Pathogen

values with different lower case letters within a N treatment or upper case letters within a pathogen between N treatments are significantly different at $P \leq 0.05$.

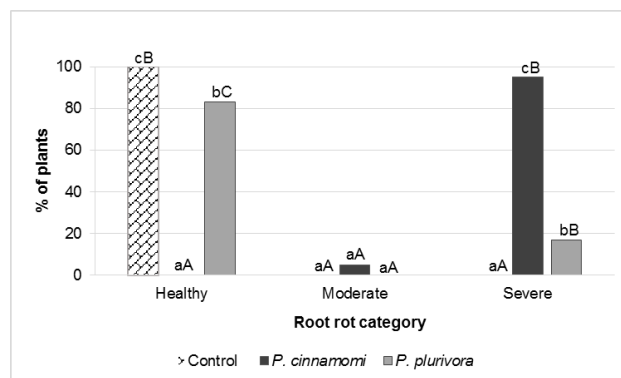


Fig. 14 Root rot categories of *Rhododendron catawbiense* 'Boursault' plants either not inoculated (control) or inoculated with *Phytophthora cinnamomi* or *P. plurivora* across both trials. Root rot was evaluated at 18 weeks after inoculation (WAI) for both trials. The root rot categories are: healthy (>50% of root system with white healthy roots), moderate (<50% of root system with white healthy roots), and severe (>75% of root system with dark, dead roots). Values are means across nitrogen treatments in both trials (n=60). Pathogen values with different lower case letters within a root rot category or upper case letters within a pathogen between root rot categories are significantly different at $P \leq 0.05$.

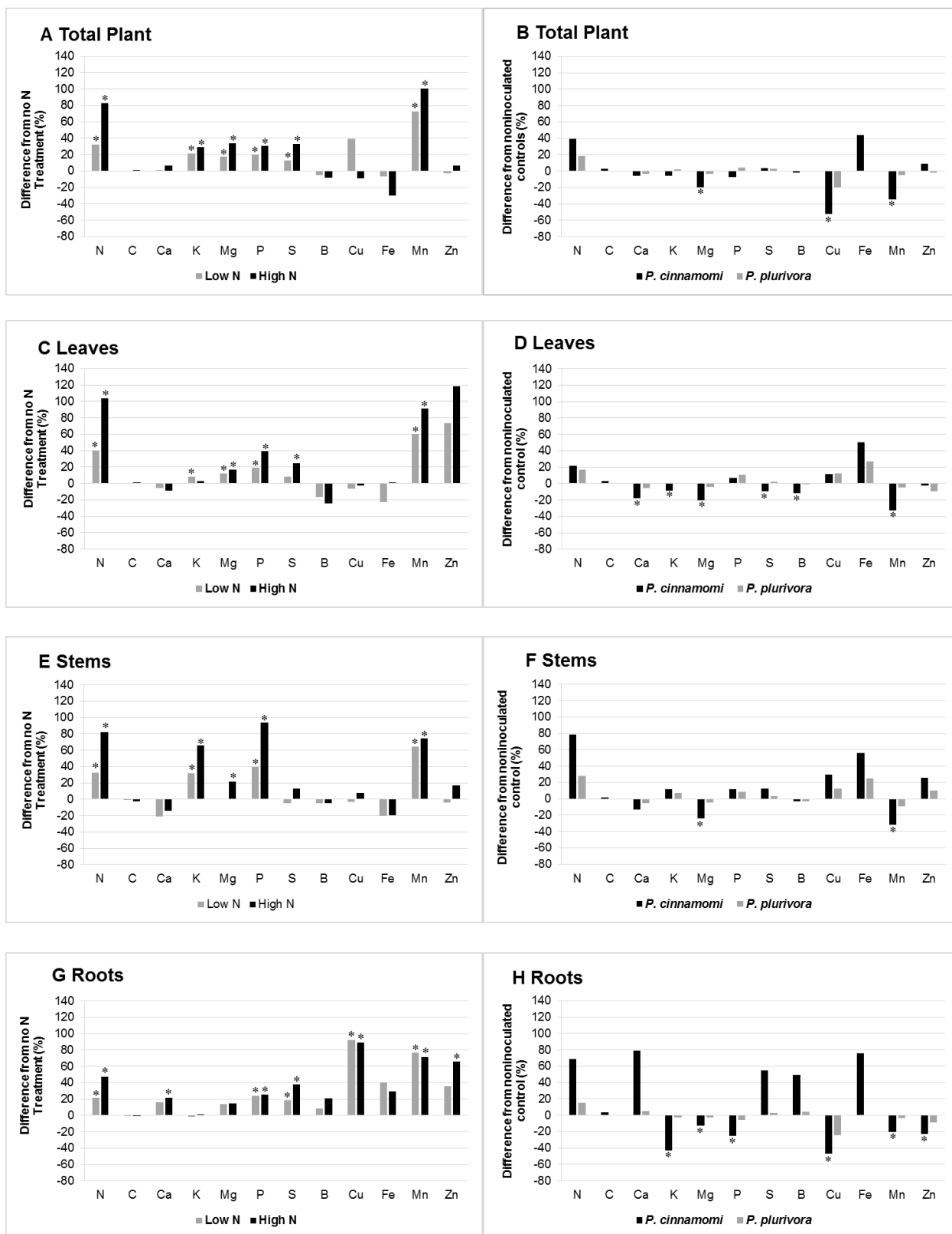


Fig. 15 Influence of nitrogen (N) application rate and pathogen inoculation on nutrient uptake (**A, B**) and leaf (**C, D**), stem (**E, F**), and root (**G, H**) composition in *Rhododendron catawbiense* 'Boursault' plants either not inoculated (Control) or inoculated with *Phytophthora cinnamomi* or *P. plurivora* and grown with three rates of nitrogen (N). At inoculation, plants received either 0 g N/pot slow release N fertilizer (No N), 1.04 g N/pot (Low N), and 3.12 g N/pot (High N). (**A, C, E, G**) Columns are relative differences in concentrations of nutrients in the low N and high N treatments compared to plants in the no N treatment. Asterisks (*) indicate nutrients in which concentration and content were greater than plants in the no N treatment. When nutrient concentration was less than plants in the no N treatment, biomass and nutrient content was similar or greater. (**B, D, F, H**) Columns are relative differences in concentrations of nutrients in the *P. cinnamomi* and *P. plurivora* treatments compared to noninoculated controls. Asterisks (*) indicate nutrients in which concentration and content were less than non-inoculated controls. When nutrient concentration was greater than controls, biomass and nutrient content was similar or lower.

Table 3 Influence of nitrogen (N) application rate and pathogen inoculation on nutrient uptake in *Rhododendron catawbiense* 'Boursault' plants either not inoculated (Control) or inoculated with *Phytophthora cinnamomi* or *P. plurivora* and grown with no nitrogen (N) fertilizer.

Nutrient	Initial ^z	18 WAI ^y		
		Control	<i>P. cin.</i>	<i>P. plu</i>
		mg per plant		
N	30	42 a*	37 a	38 a*
C	1461	4992 b*	3442 a*	4139 ab*
P	5	11 b*	7 a	10 ab*
K	20	67 b*	47 a*	58 ab*
S	5	10 a*	8 a*	9 a*
Ca	28	80 b*	54 a*	66 ab*
Mg	7	19 b*	12 a	16 ab*
		µg per plant		
Fe	357	1245 a*	1307 a*	1341 a*
Mn	544	1034 b*	691 a*	830 ab*
B	51	195 b*	114 a*	148 ab*
Cu	30	237 b*	84 a*	217 ab*
Zn	85	230 b*	155 a*	173 a*

^zInitial nutrient content the beginning of the experiment, before pre-treatment fertilizer applications (complete fertilizer without N).

^yMean values for plants grown without N fertilizer for 18 weeks after inoculation (WAI). Asterisks (*) indicate nutrients in which total plant content was significantly greater at 18 WAI than before pre-treatment fertilizer applications ($P \leq 0.05$). Means within a row followed by different lower case letters denote significant differences among pathogen treatments ($P \leq 0.05$).

Table 4 Influence of nitrogen (N) application rate and pathogen inoculation on disease progression (area under the disease progress curve, AUDPC) in *Rhododendron catawbiense* 'Boursault' plants inoculated with *Phytophthora cinnamomi* or *P. plurivora* and grown with different rates of nitrogen (N) fertilizer for 18 weeks after inoculation.

Effect ^z	AUDPC	
	Wilt	Mortality
Pathogen Treatment		
<i>P. cinnamomi</i>	468 b	699 b
<i>P. plurivora</i>	202 a	218 a
N Treatment		
No N	100 a	199 a
Low N	277 ab	399 ab
High N	627 b	732 b

^zAt inoculation, plants received either 0 g N/pot slow release nitrogen N fertilizer (No N), plants in nitrogen treatment 'low N' received 1.04 g N/pot (Low N) or , and plants in nitrogen treatment 'high N' received 3.12 g N/pot (High N).

^yAUDPC calculated based on percentage of plants that were wilted or dead. Pathogen treatment means across both trials and all N rates (n=6). N treatment means across both trials and pathogen treatments (n=4). Means within a column and effect followed by different lower case letters denote significant differences among treatments ($P \leq 0.05$).

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Chapter 4 General Conclusion

The results consistently demonstrated that *P. cinnamomi* is a more aggressive pathogen than *P. plurivora* on a number of rhododendron cultivars. This contradicts conclusions made by Weiland (2018 in press) where *P. cinnamomi* and *P. plurivora* caused similar amounts of root rot on two rhododendron cultivars. In Weiland's study, both *P. cinnamomi* and *P. plurivora* were considered aggressive pathogens of rhododendron 'Cunningham's White' and 'Yaku Princess.' Results from the current study suggest that growers should be more concerned about *P. cinnamomi* because it is more aggressive than *P. plurivora*. *P. cinnamomi* is considered to be the most important species of *Phytophthora* root rot because it is frequently isolated from nurseries and because it is the most virulent *Phytophthora* species (Hoitink and Schmitthenner 1974; Hoitink et al. 2014). However, this research does not address situations where both pathogens are present. Therefore, additional research should look at disease severity in different rhododendron cultivars that are inoculated with both pathogens.

Results also demonstrated that the experimental method of flooding inoculated plants to induce disease produces similar results as placing inoculated plants into a shallow pool of water. What this means is that the results from previous experiments that used the flooding method are probably a good proxy for the situation in nurseries where container plants sit in water. Studies have used saucers filled with water to represent standing water but have not compared the results to flooding (Tsao and Garber 1960). Given the amount of root rot damage that occurred in noninoculated

plants of the saucer treatment, however, future experiments should continue to use the flood treatment to induce disease. Growers, on the other hand, should continue to manage for reducing excess water to reduce inoculum increase and spread. Additional experiments are needed to compare the amount of damage occurring from the flood and saucer treatments to a less extreme irrigation method that is better for rhododendron health, such as an automatic irrigation system that maintains 60 to 70% container capacity, which is optimal for rhododendron growth (White 1937).

Results have shown that the amount of damage does not matter under high moisture conditions. It may be that the damage treatments were too similar to each other or that the amount of zoospores produced overwhelmed any difference between the two damage treatments. More research is needed on the effect of increasing root damage on rhododendrons grown under less extreme moisture conditions.

Results from chapter 3 showed that severity of root rot was greater with increased N in plants inoculated with *P. cinnamomi* but not *P. plurivora*. This matched previous observations Phytophthora root rot severity is greater with N in some host pathogen complexes (Scarlett et al. 2013; Tan et al. 2002; Utkhede and Smith 1995). Mortality of durian (*Durio zibethinus* Murr.) inoculated with *P. palmivora*, which causes root rot in durian, was greater with N application rate (Tan et al. 2002). Plant health of *Angophora costata* and *Eucalyptus piperita* infected with *P. cinnamomi*, decreased when fertilized with N (Scarlett et al. 2013). Severity of crown and root rot of apple trees (*Malus domestica*) inoculated with *P. cactorum* was greater when fertilized with N (Utkhede and Smith 1995). Although disease severity could be managed by reducing

nitrogen, plants exposed to inoculum would likely still develop disease if soil moisture was sufficiently high and the plants might be malnourished and undersized. A better solution would be to focus on methods that reduce contamination with pathogens and excess water conducive to spread of infectious propagules.

Overall, this research has shown the importance of pathogen species, soil moisture, and nitrogen fertility, but not root damage, on *Phytophthora* root rot severity in rhododendron. These results are important because they will allow researchers to conduct more relevant experiments and allow growers to better grow rhododendrons while reducing root rot disease.

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APPENDIX

Appendix



Fig A 1 Puddling of water at container base. Credit: Jerry Weiland.



Fig A 2 Flood (left) and saucer (right) treatment.

Table A 1 Volumetric water content of control plants in experiment one (chapter 2).

Trial	Water Treatment ^z							
	Flood				Saucer			
	Average (m ³ /m ³)	Container Capacity %	Min. (m ³ /m ³)	Max (m ³ /m ³)	Average (m ³ /m ³)	Container Capacity %	Min. (m ³ /m ³)	Max (m ³ /m ³)
1	0.36	79	0.05	0.55	0.48	95	0.36	0.58
2	0.39	83	0.08	0.72	0.51	98	0.32	0.60
3	0.40	84	0.10	0.51	0.54	102	-0.01	0.63

^zFlood: substrate saturated for 48 h 2 WAI for all trials and at 18 WAI for trial 3. Saucer: containers were placed in a saucer that was continuously filled with 1.5 centimeters of water. Plants in both the flood and saucer treatments were watered every other day with the same volume of water. Values are means from noninoculated controls averaged over all measurement times (Trial 1, n= 810; Trial 2, n=480; Trial 3, n=1074).

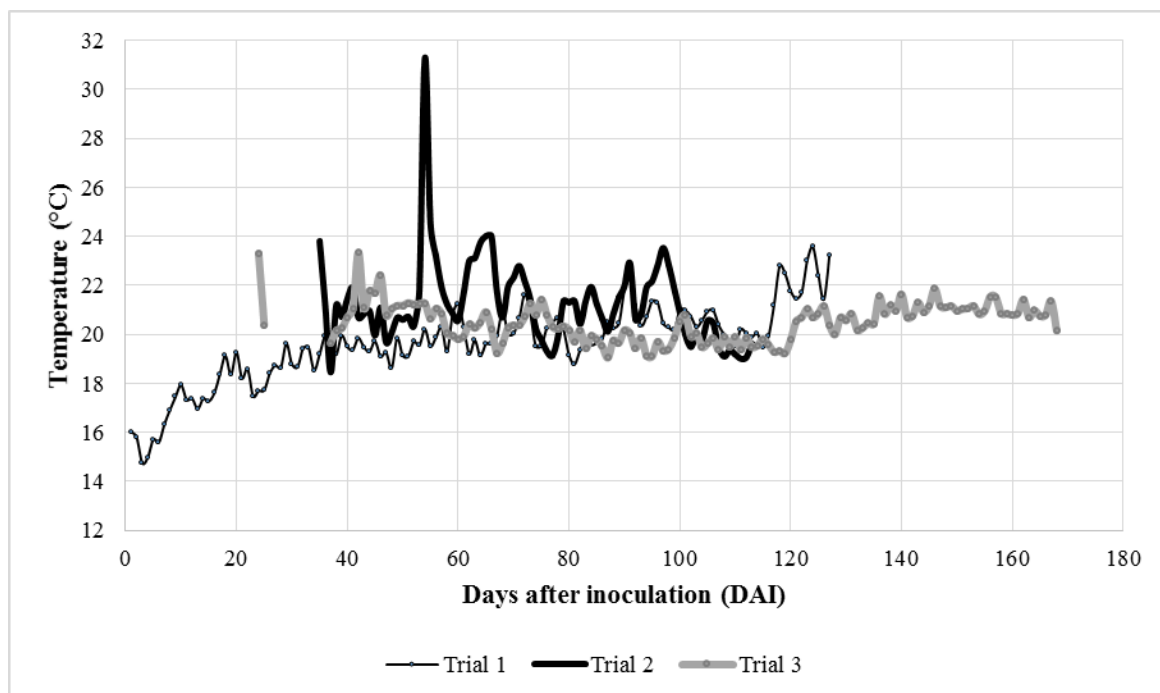


Fig A 3 Average daily greenhouse temperatures of experiment 1 (chapter 2).

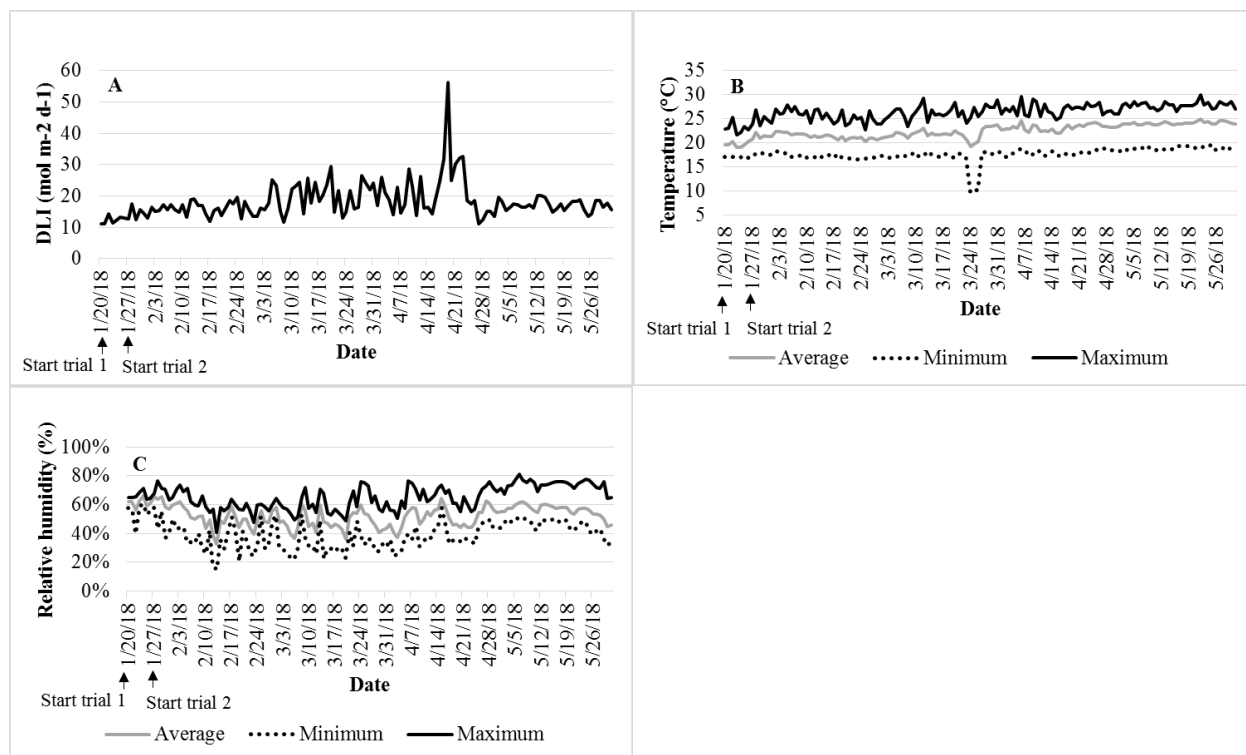


Fig A 4 Daily light integral (**A**), temperature (**B**), and relative humidity (**C**) of experiment 2 (chapter 3).

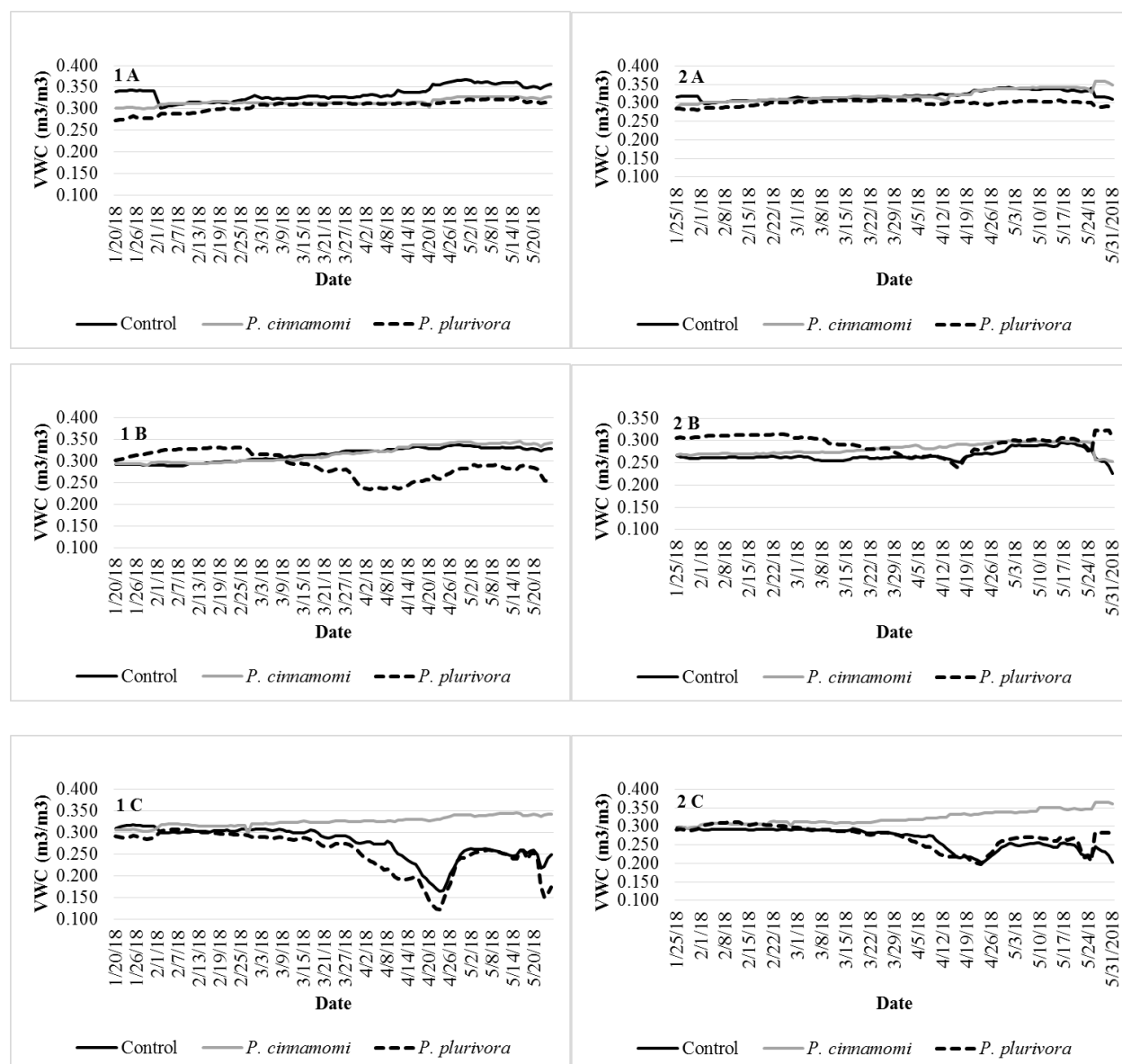


Fig A 5 Volumetric water content of *Rhododendron* 'Catawbiense Boursault' plants either not inoculated (control) or inoculated with *P. cinnamomi* or *P. plurivora* in nitrogen treatment 'no N,' in trial 1 (1 A) and 2 (2 A) 'low N,' in trial 1 (2 B) and 2 (2 B) and 'high N' in trial 1 (1 C) and 2 (2 C). At inoculation, plants in nitrogen treatment 'no N' received no slow release nitrogen fertilizer, plants in nitrogen treatment 'low N' received 1.04 g N/pot, and plants in nitrogen treatment 'high N' received 3.12 g N/pot. In trial 1, pathogen ($P=0.002$), nitrogen ($P=0.021$), day*pathogen ($P=0.013$), and day*nitrogen ($P=0.000$) was significant at $P < 0.05$. In trial 2, there was no significance of main effects or interactions at $P < 0.05$.

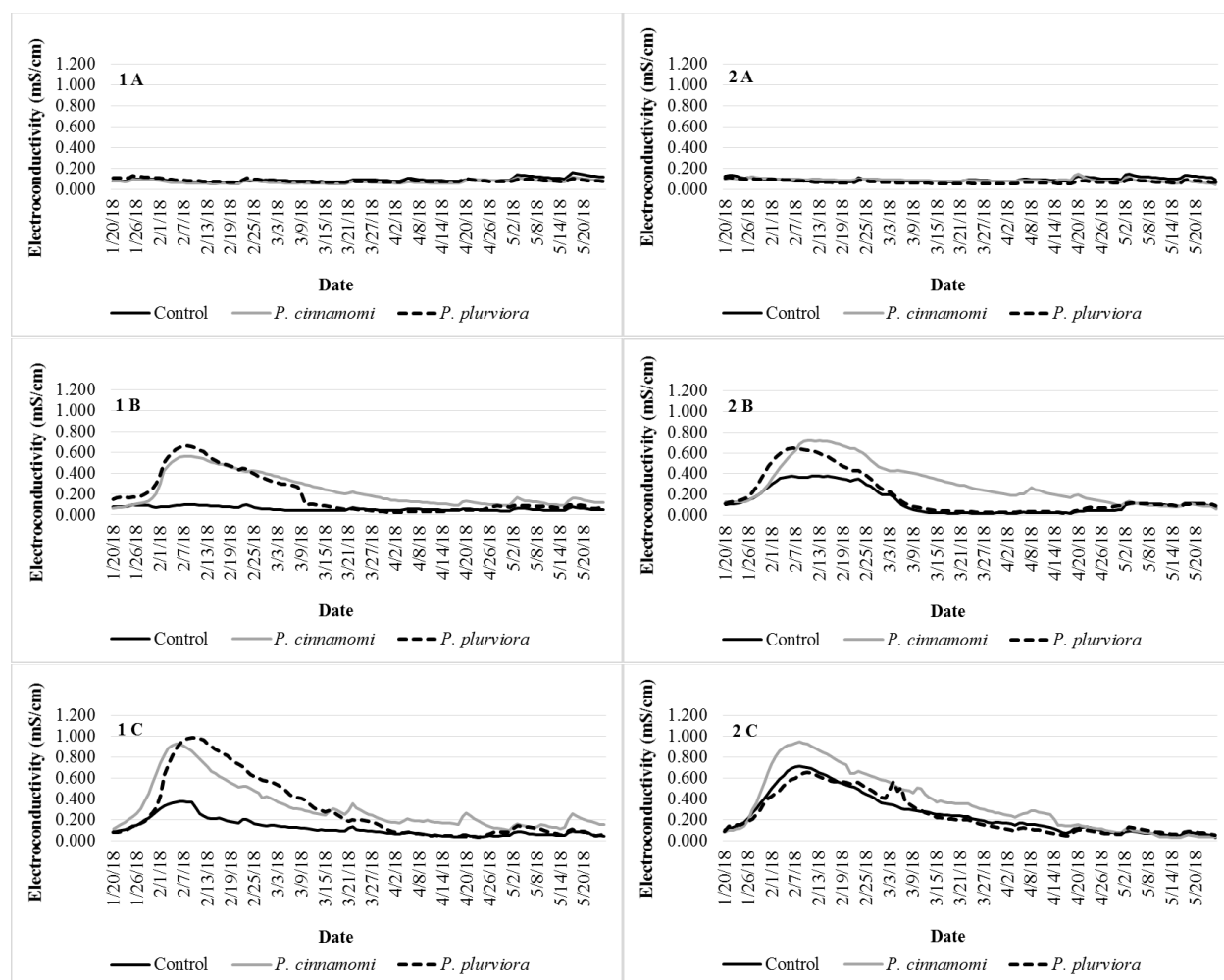


Fig A 6 Electroconductivity of *Rhododendron* 'Catawbiense Boursault' plants either not inoculated (control) or inoculated with *P. cinnamomi* or *P. plurivora* in nitrogen treatment 'no N' in trial 1 (1 A) and 2 (2 A), in nitrogen treatment 'low N' in trial 1 (1 A) and 2 (2 B), and in nitrogen treatment 'high N' in trial 1 (1 C) and 2 (2 C). At inoculation, plants in nitrogen treatment 'no N' received no slow release nitrogen fertilizer, plants in nitrogen treatment 'low N' received 1.04 g N/pot, and plants in nitrogen treatment 'high N' received 3.12 g N/pot.